IS THE USE of BREWERY SPENT GRAIN IN BIOREMEDIATION OF DIESEL CONTAMINATED SOIL SUSTAINABLE?

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DECLARATION

I wish to say that no component of the work referred in this report has been submitted in support of any application for another qualification for this or any other institutions of learning

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Abstract

Remediation of contaminated land needs to be carried out using methods that are both cost effective and minimise environmental pollution. However, the remediation option currently chosen by practitioners is often based upon limited economic information with the true environmental costs not being considered. This can result in the least sustainable option being chosen.

This study has developed a methodology to evaluate the sustainability, in terms of economic and environmental costs, for a range of treatments available for the remediation of diesel contaminated land, including bioremediation (with and without the addition of brewery spent grain), disposal to landfill and thermal treatment.

Initial laboratory investigations indicated that the use of brewery spent grain decreased the time taken for the clean-up of soil contaminated with diesel, suggesting that bioremediation augmented by the addition of this organic material was a viable option. A costing model was then developed that included all of the costs associated with the remediation options chosen. This included both direct and indirect costs. The results show that considering the indirect costs of remediation such as costs associated with delayed development the land, make bioremediation in this study an economically feasible option.

Finally environmental costs were considered with a focus on the release of carbon dioxide a known greenhouse gas. Respirometry was used to determine the volume of carbon dioxide released during the bioremediation process. This information was then combined with data collected from a range of other sources and the impact of the chosen remediation options on atmospheric greenhouse gas release was evaluated. Other environmental impacts were also determined including land and water pollution. The results indicate that bioremediation with brewery spent grain has one of the lowest environmental costs and showed that emission from pollutants such as NO_x, $PM_{1.0}$, $PM_{2.5}$, NH_3 and SO_2 could contribute to the limit values in the area covered by remediation work.

The model developed in this study has indicated that the use of bioremediation with and without the use of brewery spent grain is a sustainable remediation option providing both direct and indirect economic costs are included. The results have indicated that, the strategy of using brewery spent grain to augment bioremediation

process promotes the re-use of by-product material, reduces waste and conserve resources. There is a need for the remediation industry to adopt similar models in order that decisions made, as to the remediation option chosen, are based upon accurate costings of their sustainability.

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INTRODUCTION

1.1 Introduction

Industrialization is a hallmark of civilization however the post industrial revolution has witnessed an increase in human activities due to the emergence of the use of fossil fuels. Petroleum derived products are some of the most widely used chemicals (Sarkar *et al.* 2005) and they are often found polluting soils, water and air as a result of spills and leaks due to pipeline blow-outs, leakage of underground storage tanks, waste deposition after drilling (Xu and Lu *et al*. 2010), leakage above ground, spillage during transportation, abandoned manufacturing gasoline sites (Thapa *et al*. 2012) and current industrial activities.

These chemicals comprise a complex mixture of hundreds of hydrocarbon compounds including mixtures of non-aqueous and hydrophobic components such as n-alkane, aromatics, resins and asphaltenes (Liu *et al*. 2011). Petroleum hydrocarbon pollution represents an important environmental issue due to their toxic effects and carcinogenicity (Sayara *et al.* 2011) and these will pose serious ecological and health problems. In order to mitigate the effects of these chemicals on the environment there is a need for environmental clean-up or remediation.

Conventional methods used for the remediation of hydrocarbon polluted soils include physicochemical techniques such as vapour extraction, stabilization/solidification, soil washing, vitrification, incineration and thermal desorption (Al- Mutairi *et al.* 2008). Whilst these techniques are fairly well established they are considered to be expensive because the extracted or incinerated soil needs further treatment or disposal (Xu and Lu, 2010 and Gong, 2012). Traditionally, soils contaminated with hydrocarbons have been landfilled (Hickman and Reid, 2008) but the introduction of the European Union Landfill Directive of 1999/31 (EC, 1999) has set targets for the reduction of biodegradable waste going to landfill and has also resulted in a decrease in the number of landfill sites that will accept such waste (Hickman and Reid, 2008). Landfill has become increasingly expensive and can no longer be viewed as a sustainable option because of the high environmental footprint the

technique may leave on the environment.

As such legislative and economic drivers have driven the need for alternative options for the clean-up of polluted land and bioremediation is an increasingly popular option. Bioremediation techniques optimize the biological system already present in the soil and degrade the contaminants to an innocuous end or harmless product (Defra, 2010).

1.2 Background to the research

Bioremediation is the use of micro-organisms usually, bacteria or fungi to degrade contaminants to non-toxic by-products (Defra, 2010). The technique is thought to be a safe, reliable and environmentally benign method for the remediation of hydrocarbon contaminated soils (Nichols and Venosa, 2008). There are ranges of bioremediation techniques available including: natural attenuation, bio-stimulation and bio-augmentation (Simarro *et al.* 2013). Natural attenuation relies on the natural assimilative capacities of the soil to breakdown the contaminants present. However, the break down can be slow and uncontrolled and it relies on indigenous micro-organisms to degrade the contaminants (Kauppi *et al.* 2011). Bio-stimulation seeks to improve natural breakdown processes by the addition of nutrients or other growth limiting co-substrates not normally present in sufficient quantities in the soil (Covino *et al*. 2010). Nutrients added can be either organic or inorganic, examples include nitrogen and phosphate (Yang *et al*. 2009). The process of bioremediation can be stimulated further by the addition of a microbial consortium known to breakdown hydrocarbon pollution, bio augmentation (Covino *et al*. 2010).

Organic waste could be a source of both nutrients and micro-organisms to improve upon the breakdown of hydrocarbon pollution in soils. Utilising biodegradable by-products in this manner would divert this waste stream from landfill. Brewery spent grain (BSG) is a by-product from the brewery process that has a high water and nutrient content (Thomas and Rahman, 2006). It is currently disposed of as an animal feed. However, the amounts generated mean that the demand for the product is not as high as the volume produced.

It could be assumed that the use of BSG to improve upon the remediation of hydrocarbon contaminated soils would be an environmentally friendly technique in

that it could improve upon the breakdown of hydrocarbon pollution and also provide an economically viable disposal option for a biodegradable by-product. However, a full investigation as to the sustainability of BSG as an addition to soils, for bioremediation has yet to be carried out.

Potential environmental impacts associated with the use of BSG to improve upon the bioremediation of hydrocarbon contaminated soils include: the production of leachate from decomposing organic matter percolating through the soil and possibly into nearby water courses and the potential release of micro-organisms during the bioremediation process into the wider environment (Komilis and Ham, 2006). In addition, it may not be economically feasible to utilize bioremediation in that the cost of not developing the land, during the time taken for the bioremediation process, may be far greater than adopting the rapid method of physicochemical techniques for cleaning up the soil. In terms of the use of BSG to augment the process, there may be environmental impacts arising from transportation of the brewery by-product to the contaminated site, in addition to economic considerations.

In order to be able to make an informed decision, as to the sustainability of any contaminated land remediation option there is a need to evaluate both the economic costs, environmental costs and benefits together with the associated social issues. The main aim of this study was to evaluate the effect of adding BSG to diesel contaminated soil in the laboratory and determine the feasibility of the technique including the economic and the environmental costs.

1.3 Aims and objectives

The aim of the study reported here is therefore to answer a series of three questions concerning the use of BSG to augment the bioremediation of hydrocarbon contaminated soils:

- Does the addition of BSG improve the bioremediation of hydrocarbon contaminated soils?
- Is it economically viable?
- Finally what are the environmental costs?

Whilst the model is being developed to assess the economic and environmental

sustainability of the use of BSG in bioremediation, it is hoped that eventually it could be used for a range of contaminated land remediation options.

The aims of the study will be met through the following objectives:

- A review of the scientific literature.
- Laboratory scale investigations into the breakdown of hydrocarbon contaminants, in soil, with and without the addition of BSG.
- A full review of the costs associated with the process.
- Development and critical evaluation of an environmental cost and benefit model.

1.4 Structure of the thesis

The outline of the chapters provides an overview of this study. The thesis is structured as follows:

Chapter 2: This chapter discusses the scientific literature in an attempt to capture the background of the research. The chapter provides an historical account of bioremediation and a review of various remediation methods. Different remediation methods currently used for hydrocarbon contaminated sites have been critically evaluated. The chapter highlights the factors affecting bioremediation of contaminated land and its associate disadvantages. It describes the waste hierarchy and the role of BSG as a biodegradable by-product in the U.K. The chapter further defines sustainability in relation to the remediation sector and the role of the Sustainable Remediation Forum (SuRF) in measuring sustainable remediation in the U.K.

Chapter 3: This describes the evaluation and development of the methods used in the study, including a series of laboratory scale experiments. Method evaluation presented here covers a range of techniques such as soil preparation, experimental maintenance, microbiological methods, chemical analysis and the statistical analysis used in the study.

Chapter 4: This chapter investigates the use of BSG to augment the bioremediation process. Replicate results are presented for an evaluation of diesel breakdown in

laboratory scale experiments. The microbiology of the process has been investigated and these results are included. Chapter 4 presents the results that answer the research question ‘does the process work?’

Chapter 5: Presents the results for data collection, evaluation and analysis to determine the economic feasibility of the process. It seeks to answer the research question ‘is the process economically feasible’?

Chapter 6: Describes the development and application of environmental economics. It includes information on a method developed to evaluate CO_2 emissions. This chapter seeks to evaluate the environmental costs of a range of remediation options.

Chapter 7: This chapter focuses on the discussion of the results of the study and is divided into three sections. Each section attempts to answer the research questions posed by the study and discusses their findings. The chapter further highlights the social element of sustainability, its constraints and its importance in developing sustainable remediation.

Chapter 8: This chapter concludes the thesis and discuss the findings including the benefits of using BSG to remediate diesel contaminated soil. The chapter further highlights the recommendations for future work.

Chapter 2

LITERATURE REVIEW

2.1 Introduction

There are on-going political and social pressures to minimize the pollution arising from anthropogenic activities. Countries all over the world are trying to adapt to this reality by changing their processes to meet the challenges posed by environmental, social and economic impacts and benefits of their actions. The remediation industry is not an exception as there are different techniques that can be used to clean-up polluted land sites. Historically soil contaminated with hydrocarbons has either been landfilled or remediated by heavy engineering methods which typically offer relatively quick-fix solutions and could be expensive with high environmental and social impacts (Defra, 2010; Al-Mutairi *et al.* 2008).

Bioremediation, which is considered to be a more sustainable option, is defined 'as the use of microorganisms to remove environmental pollution from soil, water and gases' (Collins, 2001) and is often adopted for soils and sediments contaminated with hydrocarbons. Bioremediation optimizes the biological system already present in the soil and ensures that geochemical conditions such as reduction electron donor availability and oxygen content are maximised.

Legislative and economic drivers have driven the need for alternative options for the clean-up of polluted land and bioremediation is an increasingly popular option (Hickman and Reid, 2008). More so, whilst there is a general consensus on the sustainability of process-based technology, little investigation has been carried out on the relative sustainability of remediation techniques based on their wider environmental impacts (Harbottle *et al.* 2007).

This chapter will review various remediation options including their advantages and disadvantages, factors affecting bioremediation and the biological and chemical assessment of contaminated soils. It will also look at waste management in the United Kingdom with further assessments of the use of BSG and its importance in the waste cycle. The chapter will take a critical review of the sustainability of the remediation of contaminated land and the adoption of cost-benefit analysis.

2.2 Remediation of contaminated soils

The remediation industry began in the late 1970s, as a result of increasing discoveries of toxic chemicals in landfills, drinking water and traces of contaminants in urban soils (Ellis and Hardley, 2009). Since that time remediation of contaminated land has been considered to be a sustainable practice, as it enables the reuse and redevelopment of contaminated land.

Remediation involves the removal of contaminants from the environmental media this includes soil, groundwater, sediment and surface water, for the general protection of human health (Defra, 2006). The remediation of contaminated land is subject to many regulatory guidelines depending on the environmental media involved.

Remediation encourages the recovery of unused land for development purposes, urban area development, recycling of land, and minimising greenfield development (Harbottle *et al.* 2006). Hence it is an area of importance at present.

Traditionally contaminated soils were sent to landfill, however, there are on-going concerns that when these wastes are disposed of, in this manner, there can be an impact upon the environment. Breakdown of biodegradable materials, in the soils, will release carbon dioxide to the atmosphere. Levels of carbon dioxide (a greenhouse gas) have increased significantly since the early 1800's and that increase is anticipated to accelerate during the coming century (IPCC, 1995). More so, the choice of sending contaminated soils to landfill poses a danger to both groundwater and surface water as a result of leaching.

Increasingly the disposal of contaminated soils to landfill is being seen as unsustainable and increased legislation such as the European Union (EU) Landfill Directive 1993/31/EC (EC, 1999), has meant that the amounts of biodegradable wastes going to landfill must be reduced. In the U.K. the Waste Strategy has been designed to encourage recycling, recovery and composting of waste and to divert it from landfill (Harbottle *et al.* 2007; Hickman and Reid, 2008).

Despite the problems associated with landfill disposal, the quest for the swift and immediate clean-up of contaminated soil has meant that the recent focus of the remediation industry has been upon energy-intensive engineered methods (Ellis and

Hadley, 2009).

The remediation technologies reviewed in this chapter will focus upon those for hydrocarbon pollution, which is the subject of this thesis.

2. 3 Physicochemical methods for hydrocarbon remediation

The conventional methods of cleaning up hydrocarbon contaminated soils include physical and chemical methods. These methods are fairly well established and widely used both globally (Swannell, *et al.* 1996) and in the U.K. The techniques involve the destruction of organic compounds by physical or chemical means. The joint Environment Agency (EA, 2010a) publication listed fifteen types of land contamination remediation processes. The review sets out the regulatory position on different technologies that can be used to remediate contaminated soil and water (EA, 2010a). The methods are characterised as follow:

Chemical methods

- Soil flushing
- Solvent extraction
- Transformation by chemical treatment

Physical methods

- Soil vapour extraction
- Soil washing
- Permeable reactive barriers

Civil engineering methods

- Cover system
- Containment barriers
- Excavation and disposal
- Removal of groundwater for disposal/recovery

Biological methods

- Monitored natural attenuation
- Ex-situ bioremediation
- In-situ bioremediation
- Bioventing

Solidification and stabilization methods

- Solidification and stabilization

Thermal methods

- Thermal desorption

According to Defra, (2010), chemical or physical methods can be carried out onsite without excavation (in-situ) or the de-contamination of the soil can be carried out with the soil excavated above ground or removed from the site and taken to a different location for removal of the contamination (ex-situ). Chemical methods involve the use of chemical oxidants to mineralise organic pollutants from soil and groundwater. The most commonly used oxidants are hydrogen peroxide, Fenton reagent, potassium permanganate, persulfate, ozone, chlorine dioxide, reduction dechlorination and photolysis (Andreottola *et al.* 2007 and Tsai *et al.* 2009). Chemical processes may also include solvent extraction, chemical dehalogenation and surface amendment and they are introduced into the soil by various means depending on the pollutants to be removed (Semple *et al.* 2001). The chemicals are normally sprayed onto the soil allowing the solution to drain freely through the soil or by force injection (Hamby, 1996) The chemical that is used at a particular site is a function of the type of soil and the contaminants involved (Defra, 2006). This type of remediation is expensive to undertake (Defra, 2010).

Physical and civil engineering methods of hydrocarbon remediation focus mainly on capping, isolating or covering the contaminants thereby containing them and preventing their leaching into the wider environment. Physical methods that eliminate the contaminants include incineration, soil vapour extraction treatments or soil vacuum extraction systems to decontaminate or remove the contaminants. The end

products of these techniques are that the contaminants are either fixed in the soil at the site or removed for treatment to prevent dispersal into the environment (Komilis and Ham, 2006).

Although physical and chemical methods are usually adopted for soils contaminated with petroleum products including diesel, the methods are grossly inadequate with high costs (Gong, 2012). The technologies involved are regarded as expensive and technically complex. The costs of using these techniques for small scale sites are high and there is the likelihood for further contamination of the environment to occur (Vidali, 2001). In addition, landfill, and other physical methods do not totally destroy the contaminants, rather they concentrate the contaminated material in a different location (Head, 1998). Consequently, with increasing attention towards the preservation of the environment several techniques have been developed, including soil vapour extraction. Thermal desorption, is an emerging technology increasingly popular in United State of America, The Netherlands and Germany (CL: AIRE TDP24, 2010; Nathanial *et al.* 2007 cited in Defra, 2010 and William and Brankley, 2006). Another popular method in the U.K. is bioremediation, which continues to develop and mitigate the impacts of environmental problems (Greenwood *et a*l. 2009). The next sections will discuss these three methods and the problems associated with them.

2.4 Soil vapour extraction

Soil vapour extraction (SVE) is a method that involves the movement of air through the unsaturated zone to promote volatilization or biodegradation of contaminants from soil and the vapour phase (Nathanial *et al.* 2007). The technology also, known as in situ soil venting or in situ volatilization, enhances volatilization or soil vacuum extraction (FRTR, 2012). The technology entails drilling extraction wells or pipes into a polluted area, a vacuum is then applied in the extracting wells to create a pressure gradient that induces gas-phase volatile compounds to be removed from the soil. The process is described by the U.S. EPA, (2001) as the extraction wells pulling the air and vapour out of the ground, the vacuum collects them and separates the harmful vapours from the clean air and in the process the vapours sorb or stick to a solid material or they are condensed to a liquid. The solids or liquids which are polluted are then disposed of safely. But in countries such as the U.S., gases

extracted from the soil could be treated to recover or destroy, depending on the local and state air discharge regulations (FRTR, 2012).

SVE is usually used for the treatments of hazardous substances such as volatile organic compounds (VOC's), volatile metals and fuel contaminants (Defra, 2010). It has also been used to remediate other volatile and semi-volatile compounds and most gasoline constituents (U.S. EPA, 2012a). However, diesel fuel, kerosene, heating oil and lubricating oils, which are less volatile than gasoline may not be readily removed by SVE technology. The heavier products may be removed by injecting hot air to enhance the volatility (U.S. EPA, 2012a)

The SVE technique becomes necessary if the contaminants penetrate the subsurface, 5 to 15 m and have spread several hundred meters at a particular depth (Ch2m-Hill, 1985). The technique is most often considered whenever contaminants extend across a property boundary, beneath a building, or are located within an extension utility trench network where the cost of excavation and disposal may be expensive or practically impossible (Hutzler *et al.* 1988). Fig.2.1 below is a diagram of the SVE system for the treatment of volatile and semi volatile compounds.

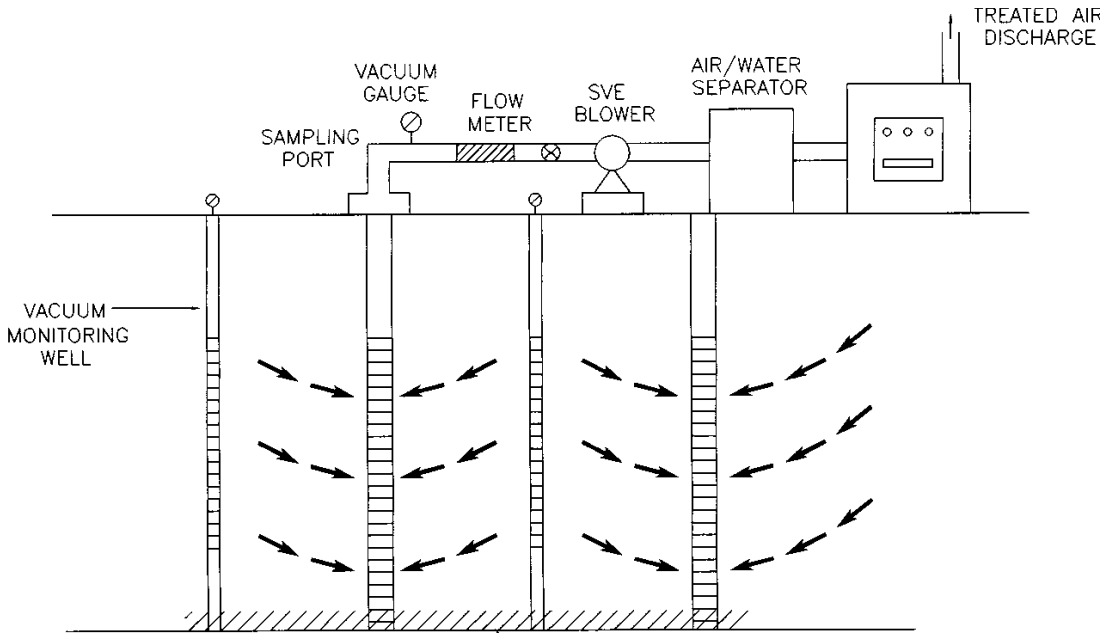

Fig.2.1: Diagram of soil vapour extraction components for capturing volatile and semi volatile compounds adapted from Suthersan (1999).

Fig.2.1 shows that SVE involves drilling one or more extraction wells into the contaminated soil to a depth above the water table. Attached to the wells is

equipment such as a blower or vacuum pump, which pulls the air and vapour through the soil up the well to the ground surface for treatment. The vacuum monitoring well monitors the pressure of the air and vapour in the unsaturated zone.

SVE treatment can be applicable in both in situ and ex situ conditions. The latter is a development of the in situ technique, the difference is that the soil would be excavated for treatment and the treatment will be on the subsurface. Air is moved through a stockpile of excavated contaminated material to promote volatilisation or biodegradation of contaminants from the soil.

The SVE process requires a system for handling off gases. If the contamination penetrates to the saturated zone or water table SVE and air sparging are often considered at the same time to clean up both groundwater and soil (U.S. EPA, 2010a). Air sparging is an in situ physical/biological method involving the injection of air (or other gases) below the water table to enhance volatilisation or biodegradation of contaminants from the soil, water and the vapour phase (U.S. EPA, 2010a). There are different types of soil vapour extraction systems which differ from each other by the method used to transfer heat to the contaminated soil, and by the gas treatment system used to treat the off-gases. The most popular method includes dual vapour extraction, dual-phase extraction or multi-phase extraction (Defra, 2010). This SVE method entails the use of a high vacuum system to remove contaminated soil or groundwater.

The SVE process requires a system for handling off gases, this is necessary because the volatile organic compounds typically present are hazardous due to their toxicity and ignitability (U.S. EPA, 2006). In most cases direct discharge of the gases without regulated treatment is unacceptable due to health, safety and public concerns. The reason for pre-treatment of gases before discharging to the ambient air is to improve the off-gas quality with minimal impact to human health or the environment (U.S. EPA, 2006). The treatment technologies for off-gas treatment are classified into four main groups by the (U.S. EPA, 2006):

- Thermal – oxidation at high temperatures, contaminants are destroyed in the vapour-phase.
- Adsorption – the process of separating contaminants using a medium

or matrix Granular Activated Carbon (GAC).

- Biological – use of living organisms to consume or metabolize chemicals in the off-gas.
- Emerging technologies – entail photocatalytic and non-thermal plasma treatment, which destroy contaminants using ultraviolet (UV) light and electrical energy respectively.

The selection of any off-gas technology depends on the nature of the distribution of the contaminants in the subsurface, the site attributes and physical properties of the chemical constituents and the overall remediation strategy. For example the thermal oxidation system could be used to treat a wide range of SVE gases and is often considered for VOC's such as petroleum products due to its reliability in achieving high destruction of the contaminants with good removal efficiency (U.S. EPA, 2006). The system can oxidize from 95 per cent to more than 99 per cent of the influent VOC (Bostrom, 2004). The three general type of thermal oxidation system include direct-flame thermal oxidizers, flameless thermal oxidizers and catalytic oxidizers.

The potential advantages of SVE include its cost-effectiveness, the technique can induce physical and biological processes, it is effective at removing many types of pollution that can evaporate, it has minimal site disturbance and can treat many organic compounds including diesel. In general, the wells and equipment are simple to install and maintain and they can reach greater depths than methods that involve digging up the soil (U.S. EPA, 2001). However, some hydrocarbons such as petroleum products and contaminants with low water solubility are harder to volatilize (U.S. EPA, 1996a). Other factors that may limit the applicability and effectiveness of the SVE process include the presence of inorganic compounds and soil with a high organic content (FRTR, 2012).

In addition, the applicability of SVE would most often depend on a field-pilot study and high costs may be incurred to collect sufficient data that is required to design and configure the system (U.S. EPA, 2012a). As well as costs associated with the process, the excavation and material handling may pose hazardous emissions to the surroundings, as exhaust air from the SVE system will require treatment to eliminate possible harm to the public and the environment. Soil that has a high percentage of

fines and high degree of saturation will require higher vacuum or hinder the operation of in situ SVE, which will also mean increased costs. In addition, due to off-gas treatment, if residuals are condensed to a liquid or solid it may require treatment or disposal, especially spent activated carbon which may need regeneration or disposal. Despite these limitations the SVE technique has been technically demonstrated and widely used and it has been selected for use in many superfund sites in the United States of America (U.S. EPA, 2010a).

The technique has less environmental disturbance, is cost-effective and could be used by facilitating extraction of higher concentrations of the contaminants when the mass removal rate has reached an asymptotic level. Other remediation measures such as natural attenuation or bioremediation can be adopted for further clean-up if the remediation objectives have not been met. The technique could be used with other methods depending on the circumstances and the remediation objective to be achieved. For example the technique has proven to be effective in conjunction with air sparging when groundwater is contaminated with evaporative compounds (U.S. EPA, 2010a).

In the U.K., soil vapour extraction is normally considered when the contaminants spread several hundred meters, extend across a property boundary, where there is a high concentration of contaminants beneath a building or in an urban area where the cost of re-location may be high. Soil vapour extraction can be applied to in situ and ex situ conditions using physical and biological methods, although its usage in the U.K has been limited (Defra, 2010).

2.5 Thermal desorption

Thermal processes use heat to increase the volatility, burn, decompose, destroy or melt contaminants (NFESC, 1998). The technology is a thermally induced physical separation process where contaminants are vaporised from a solid matrix and transferred into a gas stream where they can easily be treated (Defra, 2010). Thermal desorption is a new technology that permits on-site decontamination of the soil by heating the contaminants to a temperature level where the chemicals vaporise to a gaseous phase. This gas stream is collected or treated and even in some cases heated to a higher temperature which destroys the contaminants.

Thermal desorption can be carried out on-site (without excavating the soil) as the soil is heated to increase the removal efficiency of the contaminants, as illustrated in Fig. 2.2 and is referred to as in-situ thermal desorption (Baker *et al.* 2011). Ex-situ thermal desorption is a similar process to the in-situ method with the exception that the soil is excavated or stockpiled and introduced as a feed material. The ex-situ system involves some pre- and post-processing of the soil especially when using Low Temperature Thermal Desorption (LTTD) (U.S. EPA, 2012b). Excavated soils are first screened to remove objects greater than 2 inches in diameter, which may be crushed or shredded (U.S. EPA, 2012b). This means that the contaminated material must be excavated from its original location following some degree of material handling. Contaminated soils are excavated and transported to stationary facilities or mobile units for treatment (U.S. EPA, 2012b).

Thermal desorption has been used widely in the U.S.A. for more than 12 years to treat contaminated soil from gas plants (William and Brankley, 2006). In the U.K. there are a number of sites where thermal desorption techniques are being implemented and the regulators consider this as a viable alternative to landfill (William and Brankley, 2006). Thermal desorption is also known to be highly effective in treating soils contaminated with VOC's, SVOC's (semi-volatile organic compounds), PAH's, PCB's, pesticides, cyanides and hydrocarbons and could be applicable for in- situ and ex -situ conditions (Defra 2010).

The thermal desorption process applies heat to the contaminated media such as soils, sediment, sludge or filter cake, to evaporate the contaminants into a gas stream that is treated or managed. The treatment processes that are normally used include: condensation, collection and combustion of the gases (U.S. EPA, 1996b). The collection and condensation are usually treated off-site and combustion treatments are carried out on the site following capture of the gasses. A typical site has the following components as described in Figure.2.2 below:

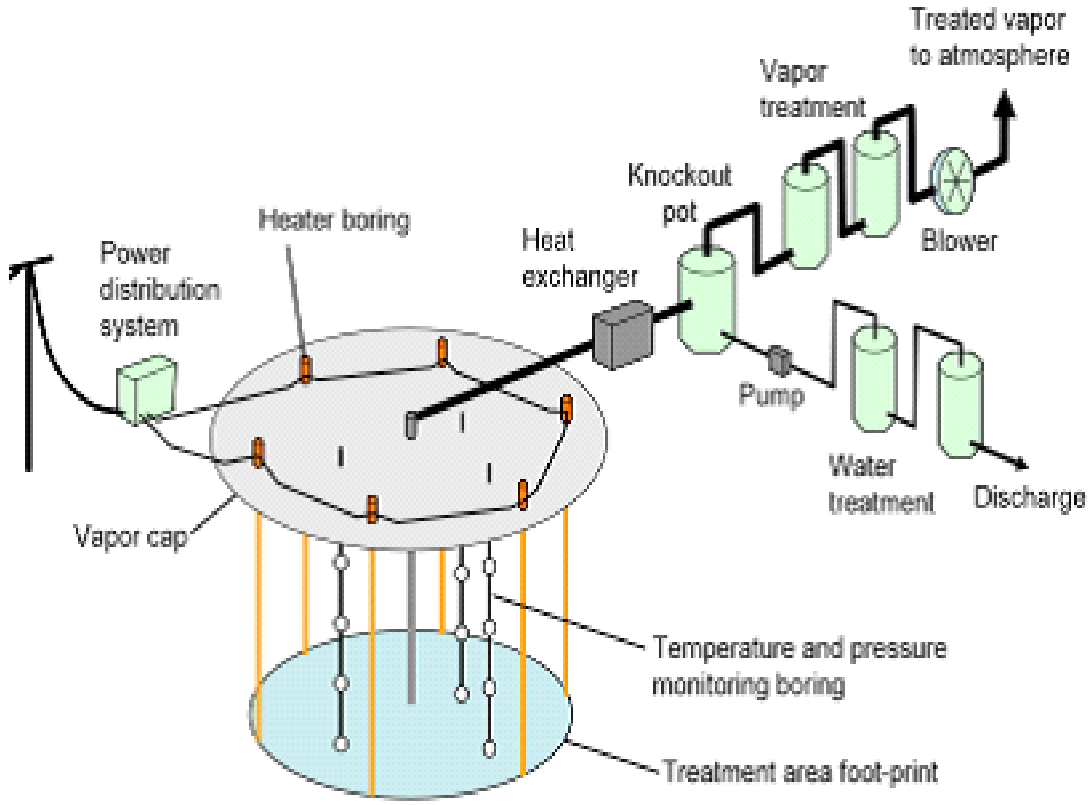

Fig.2.2 Illustration of an in-situ thermal conducting heating system adapted from Baker *et al*. (2011).

As shown in Figure 2.2 energy transfer is by thermal conduction and fluid convection around the heaters as the heater bore is heated to an appropriate temperature. The components comprise of a transformer that supplies power to the heaters, electrical distribution controller for the heaters, vapour recovery wells, temperature and pressure monitoring wells including an off-gas treatment system.

There are four main methods for in situ thermal treatment. These include injection heating (hot air), electrical resistance heating, electromagnetic heating (radiofrequency or microwave) and thermal conductive heating (FRTR, 2007; Unified Facilities; CL: AIRE TD24 cited in Defra, 2010 and U.S EPA, 2006). The efficiency of different thermal treatments will depend on applicability, the type of contaminants and soil or groundwater conditions.

The fundamental principles of thermal desorption are to increase the temperature in the ground which may promote contaminant removal by extraction or destruction. Subsurface conditions can be conducive to easy degradation or managed for

disposal. Due to different operating temperatures thermal desorption can be categorised into two groups. High temperature thermal desorption (HTTD) with contaminants heated between 600^0F to 1200^0F, and low temperature thermal desorption (LTTD) with contaminants heated between 300^0F to 600^0F (NAVFAC, 1998). For in situ low temperature thermal desorption similar to Fig.2.2 the target temperatures are in the range of 200^OC to 350^OC, depending on the physical and chemical properties of the limiting contaminant (Johnson *et al.* 2009). The applicability of the two different temperature conditions could be a function of soil type, the depth of the contaminated matrix and the type and amount of contaminants present (U.S.EPA, 2006).

The usual duration for VOC's, such as diesel oil, is between 2 months to 1 year depending on the site-specific requirements and the chosen heater spacing (Johnson *et al.* 2009) and most projects usually take 1 to 2 month for demobilization of equipment and restoration of the soil. But in a case where time is a determining factor, the chosen technology and heater spacing can be varied to match the expected project schedule at the expense of high energy usage. For instance in the U.S. this is typically carried out for brownfield sites to be developed for the construction of new homes Johnson *et al.* (2009) this is similar to the conceptual site being evaluated in the study reported here.

The technology could be appropriate for complex sites in the U.K. such as hydrocarbon contaminated sites where contaminants are not readily treatable and the technique could be used especially in cases where quicker clean-up is needed or where subsurface heterogeneities potentially limit the performance of other in situ treatment alternatives. In addition, the technology allows soil to be cleaned on site and reduces movement of vehicles transporting soils to landfill thereby saving fuel and exhaust emissions which may be an environmental concern for most remedial options in the U.K. However, the technology could be expensive as cost is driven by energy and equipment costs and both are capital intensive (operation and maintenance) and because the technique enhances the mobility of contaminants it might lead to their migration outside the treatment zone.

2.6 Biological methods for hydrocarbon remediation

Current legislation, both in the EU. and U.K., in conjunction with economic drivers

has driven the search for alternative means of remediation for soils contaminated with hydrocarbons. Bioremediation techniques have gained acceptance in the remediation industry for the clean-up of chemically contaminated land (Jorgensen, *et al.* 2000) and the technologies have been demonstrated to be feasible, quick, and deployable in a wide range of physical settings. Soil bioremediation may be broadly divided into in-situ and ex-situ techniques. According to (Xu and Lu, 2010) ex-situ techniques include landfarming, composting, bioslurry and biopilling which are carried out above ground using tilling, turning and applying oxygen and nutrients. In-situ techniques involve the same biological treatment as ex situ but without excavating the contaminated soil (Jorgensen *et al.* 2000).

The concept of the technique is based upon the enhancement of populations of microorganisms either in the soil, or added to the soil to degrade the contaminants (hydrocarbons). The technique gained popularity after the Exxon Valdez oil spill in Alaska in 1989 (Margesin and Schinner, 1997). It was reported by Pritchard (1990) that the Exxon Valdez spill was the cornerstone for a major study on bioremediation, especially through the application of nutrients such as fertilizers.

The incident has confirmed that field studies provide the most convincing demonstration of the effectiveness of bioremediation for oil polluted soils, whereas laboratory studies do not account for the numerous real world situations encountered. For example, the laboratory studies conducted by Bento *et al*. (2005) in which all three technologies of bio-stimulation, bio-augmentation and natural attenuation were evaluated and it was found that the number of diesel-degrading microorganisms and heterotrophic population was not influenced by the bioremediation treatments. Rather soil properties and the indigenous soil microbial population affected the degree of biodegradation.

In a field study conducted by Venosa *et al.* (1992) on the Exxon Valdez oil spill it was found that during a 27 day trial there was no significant difference between bio-stimulation, bio-augmentation and natural attenuation. However, In a similar study of the same Exxon Valdez spill the use of oleophilic (oil eating microbes) micro-emulsions with urea as a nitrogen source, laureth phosphate as phosphate source, and oleic acid as a carbon source and Customblen (reagent), used as nutrients for shoreline treatment and applied over 120 km of the contaminated shoreline for

approximately 2-3 weeks, was investigated. The result from this second study showed that remediation of the treated shoreline was greater than the natural attenuation shoreline (Pritchard and Costa, 1991).

The practicality of bioremediation techniques was also shown during the Gulf war in 1991, when a huge amount of oil was spilt by the Iraqi soldiers returning from Kuwait, covering about 770 km^2 of the western coast of the Arabian Gulf and about 50 km^2 of the Kuwait desert with crude oil from over 700 damaged oil wells (Cho *et al.* 1997). After the incident there were arrays of field and laboratory studies to clean up the soil and as such the efficiency of bioremediation techniques was fully tested. Radwan *et al.* (1995) carried out a field study for a period of one year with eight treatment types and their findings showed that a mixture of oil polluted soil and 3% $KN0_3$ solution proved to be most effective in reducing the extractable alkanes to about one-third of the original readings. However, repeated addition of nutrients such as sewage sludge was inimical to alkanes biodegradation due to soil acidity.

In a similar study in the laboratory using Kuwait contaminated soil Cho *et al.* (1997) applied Hyponex (soil with nutrient) and bark manure as a basic nutrient for microorganisms and applied twelve other kinds of materials and surfactants (baked diatomite, microporous glass, coconut charcoal and an oil-decomposing bacterial mixture) to accelerate the biodegradation of hydrocarbons during 43 weeks incubation. The results show that 15-33% of the contaminated oil was decomposed and amongst the materials tested coconut charcoal was seen to enhance the biodegradation of the hydrocarbon.

Since the incidents of Exxon Valdez and the Gulf war much has been achieved in the field of bioremediation both in laboratory and field studies. The incidents have prompted the development and refinement of the techniques at both a large and small scale and have been the subject of debate and research efforts in recent years. The demonstration of the significance of indigenous bacteria in degrading oil has been fully identified (Radwan *et al.* 1995). The three types of bioremediation including natural attenuation, bio-stimulation or bio-augmentation have been optimally exploited (see; Bento *et al*. 2005; Nichols and Venosa 2008; Sarkar *et al.* 2005).

Both the Exxon Valdez and the Kuwait oil spill have provided background information

on the chemistry and biological nature of contaminants. As such the use of microbial activities and chemical fingerprinting of contaminants have taken the centre stage in current and future scientific thinking. Currently, chemical data and biological techniques are adopted in assessing and describing the rate of hydrocarbon degradation. The result of biological and chemical analysis of bioremediation treatments can prove to be a useful prediction on how bioremediation should be assessed and offer transparent lines of evidence to describe degradation rates (Diplock *et al.* 2009).

Consequently, any bioremediation process must demonstrate the removal of the contaminants and the rate of degradation must be higher than leaving the soil in its original state (Bento *et al*. 2005). Hydrocarbon bioremediation can be promoted either by stimulating the indigenous microorganisms, by the addition of nutrients and oxygen or inoculation of an enriched microbial consortium into the soil (Liu *et al.* 2011; Kauppi *et al*. 2011). After several decades of studies of bioremediation there are three main approaches to hydrocarbon bioremediation: natural attenuation, bio-augmentation and bio-stimulation (Balba *et al.* 1998 and Bento *et al.* 2005).

2.6.1 Natural attenuation

Natural attenuation is the soils natural ability to degrade the contaminants without external inputs. Natural attenuation is a clean-up technology that makes use of naturally occurring microorganisms to mitigate contaminants that are present in the soil. This is referred to as intrinsic bioremediation (Moreira *et al.* 2013). Natural attenuation basically relies on the natural assimilative capacities of the soil to act upon the contaminants (Simarro *et al.* 2013) and prevent their migration. Howe*v*er, the technique is slow and uncontrolled because the method relies on indigenous microorganisms to degrade the contaminants (Kauppi *et al*. 2011).

2.6.2 Bio-augmentation

Bio-augmentation is the addition of a microbial consortium made up of bacterial species that have previously been isolated from a hydrocarbon contaminated soil (Taccari *et al.* 2012). Thus the population of microorganisms added have already been shown to be capable of removing a wide variety of hydrocarbons from the soil.

The ability of micro-organisms to degrade hydrocarbons has been known for many

years and Zobell, (1946) identified over 100 microbial species from 30 genera that could degrade some type of hydrocarbons. These organisms are found in the environment and are widely distributed in fresh and salt water, soil, and groundwater (Borden *et al.* 1995). In practice the technique of bio-augmentation involves the introduction of microorganisms that have been selected, for their ability to breakdown hydrocarbons, and then cultured in the laboratory to increase numbers before being added to the contaminated soil. These organisms may have been derived from the existing contaminated soil or could have been obtained from a stock of microbes that have been previously certified to degrade hydrocarbons. Once the microorganisms enter the soil they selectively consume the hydrocarbons (Sarker *et al.* 2005) and use them as a source of energy (Huang *et al.* 2013).

The bio-augmentation process may be enhanced by the addition of nutrients essential for microbial growth, if soil analysis shows them to be lacking (Tsai *et al*. 2009). Nutrients commonly added are nitrogen and phosphorus (Gong, 2012). However, the use of genetically engineered microorganisms, for use in bioremediation, has seen little development over the past decade (Sayler and Ripp, 2000) due to ethical considerations and a lack of motivation from regulatory agencies (Miller, 1997).

2.6.3 Bio-stimulation

Biostimulation is the introduction of nutrients in the form of organic and/or inorganic materials into the soil. Natural attenuation is often limited due to the absence of essential nutrients needed by the micro-organisms for growth; bio-stimulation is the addition of these nutrients to optimise bacterial growth. The nutrients added could be either organic or inorganic such as nitrogen and phosphorus (Sarka *et a*l. 2005) or other growth-limiting co-substrates (Nikolopoulo *et al.* 2013). Bio-stimuation techniques have been shown to improve the biodegradation of oil under Antarctic conditions and proved to be beneficial using nutrients and best experimental design, constituting a promising alternative for some hydrocarbon-contaminated Antarctic soil restoration (Dias *et al.* 2012).

The concept of bio-stimulation is that the addition of nutrients into the soil will stimulate and increase the population of indigenous microorganisms. According to Sarker *et al.* (2005) the indigenous microorganisms may or may not remove the

hydrocarbons from the soil or use it as a source of energy, but it is assumed that the degradation occurs more quickly in comparison with natural attenuation as a result of increased numbers of microorganisms due to increased levels of nutrients.

2.6.4 Factors affecting bioremediation

The environment exerts a significant influence on microbial activities and whilst micro-organisms are present in any contaminated soil (Lee *et al*. 2012) the availability of contaminants to the micro-organisms is influenced by a range of biotic and abiotic factors. Factors that influence microbial degradation include: the indigenous microbial populations; nutrient availability; oxygen; pH; temperature; water content; bioavailability of contaminants and soil properties (Margesin and Schinner, 1999; Taccari *et al*. 2012). During the bioremediation process the optimisation of these factors will lead to the effective degradation of pollutants.

1. Temperature: This is an important abiotic factor for successful bioremediation of contaminated land. Temperature exerts an influence on the nature and extent of microbial hydrocarbon metabolism (Chang *et al*. 2010). In many temperate and cold climates the degradation of aliphatic hydrocarbons is hindered by low ambient temperatures during most of the year (Ferguson *et al.* 2008). Thus, some environments are associated with extreme temperatures either in artic or semi-arid regions. The U.K. is associated with cold temperatures during the winter and this may limit the use of bioremediation.

In colder regions the rate of hydrocarbon degradation is seen to be very low and as such the removal of hydrocarbons from the soil may be difficult and the contaminants may remain in the ecosystem over a long period of time. In a study of bioremediation of hydrocarbon contaminated soils in cold temperatures, such as the artic region Whyte *et al.* (1998) noted that there was an increase in the viscosity of the oil in cold climates and this reduced the movement of the oil through the soil thereby reducing the chance of micro-organisms acting upon it.

However, there are cold-adapted indigenous hydrocarbon degraders in cold artic regions because there are certain bacteria species that able to grow and metabolize hydrocarbons over a wide temperature range (Chang *et al*. 2010). But a comparison of oil decontamination at 10^0C to results reported at 25^0C – 30^0C underscores the

effectiveness of cold –adaptive degraders (Margesin, 2000). This shows a correlation between decreasing temperatures and decreasing rates of enzymatic activities, which are responsible for oil degradation. High temperatures tend to favour the use of thermophilic bacteria for biodegradation of hydrocarbons. High volatilization and solubility of some hydrocarbons at optimal temperatures influences toxicity and allows biotransformation at high substrate concentrations (Whyte *et al*. 1998).

2. Oxygen: Most petroleum hydrocarbons are biodegradable under aerobic conditions (Thapa *et al.* 2012). Oxygen can be seen as a co-substrate for enzymes that catalyse the metabolism of hydrocarbons (Young, 1984). During bioremediation, hydrocarbons are known to be oxidized (donate electrons) and an electron acceptor (e.g oxygen) is reduced (Borden *et al.* 1994). There are a number of other known electron acceptors including nitrate, iron oxide, water, sulphate and carbon dioxide. In the bioremediation process if oxygen is depleted microorganisms may use other oxidizing agents as an electron acceptor. However, oxygen is the preferred electron acceptor due to microbial ability to obtain more energy from aerobic reactions.

3. Nutrient availability: The addition of nutrients to soil is to increase soil structure and optimise the moisture content (Chang *et a*l. 2010 and Abioye *et al.* 2012). Nutrients commonly added are inorganic such as nitrogen and phosphorus (Chang *et al*. 2010). But as the field of bioremediation continues to expand the application of organic compounds becomes more popular in order to aid the metabolism of soil microbes. In recent times bulking agents such as wood chips, vermiculite, animal droppings, compostable municipal waste, poultry manure and other green wastes are now used as nutrient supplements in the bioremediation of contaminated soil (McMahon *et al.* 2008; Adesodun *et al.* 2007 and Defra, 2005).

4. Bioavailability of the contaminants and Indigenous microorganisms: One of the challenging tasks of effective bioremediation is the ability of microorganisms to degrade the contaminants to a residual point where it can be considered harmless to the environment. The indigenous microorganisms are known to degrade hydrocarbons more effectively than the introduced strains. This is because the introduction of external oil degrading bacterial or known hydrocarbon degrading bacteria may take a longer time to acclimatize to the contaminated environment.

In a laboratory experiment conducted by Cho *et al.* (1997) using contaminated soil

from the Kuwait desert, of the ten treatments they studied, the samples with oil decomposing bacterial mixtures impeded the bioremediation process. In another study by Radwan *et al.* (1995) using the same Kuwait desert contaminated soil, the introduction of oil degrading bacteria was not effective in the removal of the hydrocarbons from the soil whilst the optimization of indigenous oil degrading bacteria demonstrated a significant reduction of hydrocarbons from the soil.

More so, in a study conducted by Kim *et al.* (2005), where three treatments including the application of surfactant, oil-degrading bacteria and monitoring of indigenous bacteria were evaluated it was found that the application of oil-degrading bacteria in a concentration higher than the indigenous microbial populations significantly increased the clean-up process. These finding's to some extent emphasize the role of dominance in microbial populations in terms of remediating polluted soils. The indigenous organisms are known to have a niche within the hydrocarbon contaminated site because they use the carbon as a source of energy. As such if external bacteria are introduced they must be in a large quantity and have the ability to dominate their indigenous counterparts. However, if the remediation entails a large area the cost and complexity of using external bacteria could involve a huge resource. There may also be regulatory concerns about the use of genetically modified organisms.

5. Soil properties, pH, and water content: Environmental parameters such as temperature, pH, moisture content, soil properties, quantity and bioavailability of contaminants and redox potentials are significant for bioremediation (Fountoulakis *et al*. 2009 and Margesin, 2000). The ability of the micro-organisms to adapt to the environmental properties of the soil is a function of the nature and activity of the soil bacterial community.

However, the modification of the environmental parameters could enhance bacterial growth or survival. For example the measurement of pH in a soil is important to understand the types of microbial species within that soil. This is because each species of bacteria have their optimum pH value for growth. Thapa *et al.* (2012) asserted that microbial growth and activity are readily affected by pH, temperature and moisture. More so, soil pH may depend on other environmental factors such as mineral and water content.

One important soil property in the bioremediation process is the value of moisture content. Thus, water is an essential component in cell processes as it aids the transportation of nutrients to the microbes. It also, serves as a medium for microbial growth and regulates soil temperature. The water content in a soil can be determined by measuring the soil water holding capacity.

Another dominant feature of soil properties is the clay-organic matter complexes. The aggregation of the clay and organic matter complexes is an important element governing microbial activities in soil matrices (Paul and Clark, 1998). The composition of the clay-organic matter determines the availability of water infiltration, oxygen tension and nutrient movement as required by microbial communities.

2.6.5 Disadvantages of bioremediation

In spite of the increased number of research studies investigating the use of bioremediation, it has yet to be accepted as a routine treatment technology and the environmental industry including the legislature are still wary of adopting the method for the treatment of sites contaminated with hazardous chemicals. If bioremediation offers benefits over other technologies, why has it not been more widely adopted to treat environmental contamination?

One reason often put forward is that the physiochemical treatments are quick and their outcome is predictable in the short term (Defra, 2010). More so, they are relatively inexpensive considering the time frame required for bioremediation. According to Juhassz *et al.* (2000) one of the difficulties of developing bioremediation strategies lies in achieving as good or better results in the field as in the laboratory.

In addition there are numerous studies in the field of bioremediation that have shown that out of the thousands of chemical compounds, in oil, only a few fractions are known to be susceptible to biological degradation. Several bacteria and filamentous fungi have been reported to detoxify and degrade hydrocarbons (Borras *et al*. 2010). Fractions of hydrocarbon that have been shown to be biodegradable according to Huang *et al.* (2013) includes; alkenes, aromatic hydrocarbons and polycyclic aromatic compounds. These compounds are used by hydrocarbon degrading microorganisms as carbon sources, breaking them down into fatty acids and then into carbon dioxide (Prakash and Irfan, 2011; Haritash and Kaushik, 2009 and

Huang *et al*. 2013).

However, in a study conducted by Serrano *et al.* (2010), it was found that the addition of a mixed bacterial consortium (Bioaugmentation) did not significantly affect the biodegradation efficiency for polycyclic aromatic hydrocarbons (PAH's) with low molecular weights. Similarly a field study carried out by Garcia-Blanco *et al*. (2001) on the shoreline of the St. Lawrence River found that the augmentation of nutrients (biostimulation) did not have any major effects on the clean-up processes especially when the oil had permeated significantly into the sediment.

In any bioremediation process the rationale for the application of exogenous bacterial to the soil may be that the indigenous bacteria may not have the ability to degrade the contaminants. But the importation of cultured bacteria strains is assumed to lead to improved degradation, as the added culture are able to survive and express their oil-degrading ability in the environment to which they are applied. Bioremediation has been described as a multi-variable process, and the optimization of the process through classical methods was inflexible, unreliable and time consuming (Huang *et al*. 2013). One of the arguable shortcomings of bioremediation is the unpredictability of the process, due to a lack of understanding of the behaviour of microbial populations in natural environments and how physical, biological and chemical factors interact to control their ability to breakdown environmental pollutants.

One of the difficulties associated with bioremediation is the inability to effectively evaluate the biodegradation process of the different bioremediation techniques due to physical and chemical weathering, which may significantly affect the composition and concentration of oil contamination (Nichols and Venosa, 2008). This could be as a result of the polluted area being exposed to the contamination over a long time period or at a newly polluted site the oil could be lost due to volatilization, adsorption to organic compounds and other abiotic factors that are equally involved in the reduction process (Adesodun and Mbagwu, 2008).

2.7 Monitoring and measurement of bioremediation

Evaluating the success of bioremediation may involve chemical, microbiological and ecotoxicity assessment of soil polluted by oil, in order to improve the understanding

of the pollutants, microbial community dynamics and ecotoxicological effects of various bioremediation strategies (Llado *et al*. 2012). Oil has been described as a highly complex assemblage of organic compounds that no single analytical method can entirely characterise (Al-Mutairi, *et al*. 2008). Hence the measurement of the effectiveness of the bioremediation process may be inaccurate. It has been suggested that the efficiency of the bioremediation process should be evaluated using a chemical and toxicity test both during and post-remediation (Al-Mutairi *et al.* 2008). Llado *et al.* (2012) also state that acute toxicity tests would confirm the improvement in the quality of the soil to give an insight into the progress of bioremediation strategies.

Toxicity testing during bioremediation and post-bioremediation may serve as an indicator of soil quality and the potential risk the contaminants pose to the natural ecosystem (Margesin and Schinner, 1997). In a field study conducted by Salanitro *et al.* (1997), evaluation of earthworms, seed germination and plant growth were used to monitor total petroleum hydrocarbon (TPH) degradation. Their findings showed that remediated soils were not toxic to earthworms and did not inhibit seed germination after 10-12 months of treatment. The study showed that contaminated soils could lose their toxicity and the potential to leach contaminants if successfully bioremediated.

In recent times the measurement of hydrocarbon concentration by crop yield has opened a frontier in the field of bioremediation, as plant growth has successfully been used to measure the remediation of contaminated soils rather than as a measure of soil toxicity. This emerging technology in many literatures has been referred to as phytoremediation (Moreira *et al.* 2013). Phytoremediation is a low-cost process based on microbial activation in the root zone and is regarded as a novel method employed to remediate contaminated soil with VOC's and SVOC's (Defra, 2010).

At present there is no evidence of a universal TPH clean-up standard that can be adopted, rather the efficiency of the clean-up is assessed using criteria based upon risk-based assessments coupled to an evaluation of the environmental fate and effects of the pollutants (Salanitro *et al*. 1997). In the United Kingdom the contaminated land regime which is set out in part IIA of the Environmental Protection

Act 1990 (DETR, 2000) provides a risk based approach to the identification and clean-up of sites which are considered to be contaminated. The risk to humans or the wider environment is evaluated and land is only considered to be contaminated if there is a source, pathway and receptor. The part IIA regime bestows the responsibility for the identification and clean-up of contaminated land on local authorities as the primary regulators (DETR, 2000). Appropriate remediation will prevent, minimize or mitigate the effects upon human health and the environment. In terms of hydrocarbons the commonly used measure under the U.K. regime is chemical and biological assessment.

Most monitoring studies for bioremediation rely on the outcome of chemical analyses such as gas chromatography or mass spectrometry to evaluate efficacy and infer residual oil toxicity (Barthe, 1986). Therefore, there is a range of both chemical and biological techniques that could be used to measure the efficiency of hydrocarbon degradation both during and post-bioremediation. If the chemical and biological assessment proved a degrading rate of contaminant concentration, then the timeframe for the target to be achieved can be predicted.

The timeframe for a bioremediation objective to be achieved depends on the various factors described (section 2.6.4.) above. In the review of Maila et al. (2005) the success of bioremediation of TPH sites was noted in addition to their efficiency in terms of percentage degradation of the TPH. Table 2.1 shows the duration of various treatments of biostimuation and bioaugmentation which ranges from 3 months to 3 years depending on the treatment and contaminant types.

	Efficiency (%) of oil removal	Microbial process including the pollutant	Duration	References
Bioremediation techniques	11	Bio-augmentation	60 days	Lee *et al.* 2011
	36.28%	Bio-stimulation	42 days	Ameh *et al*. 2012
	83-93	Bio-stimulation PAH's	161 days	Greenwood *et al*. 2009
	78	Bio-stimulation PAH's	90 days	Picado *et a*l. 2001
	75	Bio-stimulation	118 days	Benyahia *et a*l. 2005
	83	Bio-stimulation	126 days	Dadrasnia and Agamuthu, 2012
	92	Bio-stimulation	84 days	Abioye *et al.* 2012

Table 2.1: Reported success rates for bioremediation of TPH from different sites using bio-stimulation and bio-augmentation including the percentage degradation and duration of the bioremediation process

Table 2.1 reports the results of studies into the bioremediation of hydrocarbon contaminated land both in the U.K. and globally, which have indicated a period of 3 months to 3 years for the bioremediation objective to be met. Also in a study conducted by PWTB (1999) in which samples from a variety of military operations in Washrack were incubated and analysed, it was found that degradation levels recorded met the reuse of soil level requirements in 6 months.

Another study in the U.K. where former gasworks sites owned by British Gas were remediated (volume of treatable material 33,534 m^3) the contaminants including hydrocarbons were degraded and the site clean-up target was achieved in 6 months, for all contaminants, at a trigger level recommended by the Environmental Agency (Day *et al*. 1997). Another study that confirmed the efficacy of the bioremediation process was the fieldwork conducted by Picado *et al*. (2001) in Portugal in which the overall soil treatability of hydrocarbon contaminated soil detected a reduction of

PAH's with 2,3,4 rings by 79 % within the period of 3 months.

The use of micro-organisms to remove hydrocarbon pollutants has been established for decreasing the concentration of contaminants and reducing the risk to human health and the environment. However, the use of the technique as a remedial strategy at contaminated sites is still unpopular amongst remediation practitioners. There are though strong drivers to increasingly adopt bioremediation as an effective method to mitigate hydrocarbon contaminated soils (Diplock *et al.* 2009).

As such, chemical and biological data could be used to evaluate the efficiency of the technique and could provide the regulatory body the metrics required to adopt the technique as the most effective method to monitor oil contaminants from aquifers. More so, assessment should provide a reasonable time frame for the remediation objective to be achieved and should offer a transparent line of evidence to explain degradation rates including the endpoint of the remediation target. Thus, laboratory experiments must be effectively extrapolated to the field scale and biological assessment and quantification of the bioavailability of hydrocarbons to bacteria, degraders and heterotrophs should allow the evaluation of how bioremediation would be measured (Diplock *et al.* 2009).

Therefore, the next section contains a review of some commonly used approaches, some of which will be adopted to test the efficacy of the bioremediation technique being evaluated in this study.

2.7.1 Biological techniques

Extensive efforts have been made in the field of bioremediation to investigate the role of enzymatic reactions during the degradation of contaminated soil (Kaplan and Kitts, 2004; Perry, 1984; Salminen, 2004; Venkatesan and Senthurpandian, 2006; Zhang et al. 2006). Soil enzyme activities could then be used as indicators of contaminant degradation as it incorporates information about microbial status and the physicochemical condition of the soils (Chen, *et al.* 2003).

In the field of agriculture soil enzymes are often used as a biological marker of soil fertility (Venkatesan and Senthurpandian, 2006) and the measurement of enzyme activity in contaminated soils could also be used as a measure of changes in the composition, structure and activity of microbial populations (Kaplan and Kitts, 2004).

The mechanisms underpinning the degradation of hydrocarbons, in the soil, are the stimulation of growth and activity of hydrocarbon degrading micro-organisms in the rhizosphere (Frick *et al*. 1999). Enzymes such as phosphatases, proteases, ureases and pectinases secreted extracellularly by the micro-organisms into the soil could be used as a measure of microbial activities (Venkatesan and Senthurpandian, 2006).

Measures such as microbial biomass and enzyme activity could be used to assess both the health of the soil and the efficiency of the bioremediation process (Dick and Tabatabai, 1992). Microbial plate counts on differing media will give an indication of the populations of micro-organisms present whilst soil enzyme assays will give an indication of microbial activities. Enzyme assays commonly used to assess the efficacy of bioremediation include proteases, phosphatases, soil respiration and dehydrogenases.

1. Protease Enzymes

Proteases are degradative enzymes that catalyze the hydrolysis of proteins (Ali and Dahot, 2009). They are produced by a range of bacteria, actinomycetes and fungi (Glenn, 1976). Protease activity is directly affected by the availability of carbon and nitrogen (Geisseler and Howarth, 2008). The activity of protease has been described in the literature. Kumar and Takagi (1999) asserted that soil proteins are hydrolysed to peptides and amino acids by extracellular proteases. While Ali and Dahot, (2009) described proteases as degrading enzymes that hydrolysed nitrogen compounds to ammonium using urea and low molecular weight protein substrates.

Proteases have been extensively used in numerous fields but in bioremediation protease activity could provide a better understanding of the biochemical processes occurring in soil and demonstrate how biological parameters possess the potential to act as sensitive indicators of soil ecological stress and restoration.

2. Phosphatase enzymes

The absorption of phosphate from organic compounds by micro-organisms and plants is catalysed by the enzyme phosphatase. If concentrations of hydrocarbon contaminants are high in the soil, phosphate is known to be a limiting nutrient for microbial growth during bioremediation (*URL1*) hence concentrations of phosphatase may be low. In the field of bioremediation it has been used to determine when

indigenous microbial populations are deficient in phosphate.

3. Dehydrogenase enzymes

Dehydrogenase enzymes are vital in the oxidation of organic matter in that they transfer hydrogen from the organic substrate to the electron acceptor (Balba *et al.* 1998). More so, dehydrogenase may be used to measure process performance by monitoring changes in the composition of the contaminants and biochemical process parameters during bioremediation (Gong, 2012).

4. Soil respiration

In soil ecology, soil respiration is increasingly used for various purposes including the diagnosis of the state of the soil in agricultural management. Respiration analysis provides information on the biochemical processes occurring in the soil and is seen as an important ecosystem process that releases carbon from the soil in the form of carbon dioxide. Abiotic factors such as temperature, moisture and nutrient content, as well as the level of oxygen uptake in the soil can all dictate the rate of soil respiration.

In studies of bioremediation the possibility of obtaining basic information on the biodegradability of contaminated soils, in terms of the supply of carbon, for microbial growth and metabolism are well established (Nelson et al., 1996; Pagga *et al.* 1995). Another study by Gomez and Sartaj (2013) measured the CO_2 concentration inside biopiles along with TPH percentage degradation. The results suggested that there was biological activity inside the biopiles, confirming the successful adaptation of the inoculated bacteria in the soil, which improved the efficiency of hydrocarbon degradation.

Jorgensen *et al.* (2000) evaluated three different composting treatments using bark chips, two commercial microbial inocula and nutrient amendments, in a field scale trial, on a diesel oil contaminated soil. Three different treatments were evaluated by measuring soil respiration rates. It was found that the treatment with the addition of large amounts of organic matter produced more CO_2 than the treatment with mixed microbial inocula. Respiration as with enzyme activity can detect microbial activity in the soil. It could therefore be used as a measure of the breakdown of hydrocarbon contamination.

5. Microbial enumeration

In studies investigating the bioremediation of contaminated soil microbial counts are normally carried out in order to determine both the numbers and types of microorganisms present in the soil. They can be used to distinguish between fungi, bacteria and actinomycetes (McMahon *et al.* 2008).

During bioremediation the identification of hydrocarbon degrading bacteria through microbial counts can provide useful knowledge on soil biological activities and the extent to which the indigenous microbial populations have acclimated to the site conditions (Balba *et al.* 1998). The counts will also show if the soils have indigenous microbial populations with the potential to support the bioremediation process. The growth media most often used are mineral agar, malt agar, chelate mineral media and R2A agar.

The field of bioremediation is expanding and over the past decade progress has been made in terms of the identification of the various bacteria genera capable of degrading hydrocarbon compounds at the field scale. Simarro et al. (2013) studied the decontamination of creosote contaminated soil through microcosm assays, evaluating changes in the bacteria community and toxicity. They characterised the microbial population using deoxyribonucleic acid (DNA) extraction, molecular and phylogenetic analysis.

The identification of species of bacteria with the aid of culture-independent molecular biological techniques offers a new opportunity to better understand the dynamics of microbial communities. Nucleic acid based molecular techniques for fingerprinting the 16S ribosomal DNA of bacteria cells for the detection, description and enumeration of bacterial targets has gained wide attention in modern microbial ecology (Morales and Holben, 2009) and this approach is sometimes followed by the sequencing of the gel bands to isolate the bacteria that are directly related to the degradation of contaminants (Ciric *et al.* 2010).

2.7.2 Chemical analysis

The development and advancement in chemical fingerprinting and data interpretation technique provides the opportunity for petroleum mixtures to be fractionated by chromatography. The techniques have been used to monitor the distribution pattern

of petroleum hydrocarbons, analysis of source-specific marker compounds, diagnostic ratios of specific oil constituents (Wang *et al.* 1999) and determination of TPH levels in the soil (Moreira *et al.* 2013). There are a variety of techniques used to screen soils contaminated with hydrocarbons including diesel, PAH's and other petroleum constituents, however, the use of chromatography has become popular. This technique entails the separation of components in a mixture by partitioning between a mobile phase and stationary phase. The types of chromatography commonly used to screen or measure hydrocarbon constituents are:

2.7.3 Gas chromatography (GC)

This is a method for separating a mixture of compounds by partitioning the components between a flowing gas (mobile phase) and non-volatile liquid phase (stationary phase). GC is the most popular method and it is often used to screen sediments for petroleum saturated and aromatic compounds and measure TPH. GC can be used to determine the presence of petroleum compounds in a soil or water and to qualitatively measure and examine their degradation rate (Wang et al., 1999). GC has detectors such as the flame ionisation detector (FID), which is used to measure TPH and an electro chemical detector (ECD), which is used to measure chlorinated species. The GC-FID does provide a description picture of the major oil components with the capability to monitor the effect of microbial degradation on the loss of hydrocarbon at contaminated sites.

Another method is GC coupled with mass spectroscopy (GC-MS). The GC uses the mass spectrometer to detect the components separated in the column. It can be used to measure a sample in order to determine the pattern representing the distribution of components (atom-molecules) by mass (mass to charge ratio). The MS technique operates by bombarding a sample with electrons within a chamber under vacuum. Then the molecules of the compounds become positively charged due to the loss of electrons, forming fragmented ions, these fragmented ions pass through a magnetic field where they are separated according to their mass. GC-MS has a capillary that is capable of analysing the oil-specific biomarker compounds and polycyclic aromatic hydrocarbons.

Other advanced methods for analysing hydrocarbon constituents are infrared spectroscopy (IR), supercritical fluid chromatography (SFC), ultraviolet, isotope ratio

mass spectrometry and gravimetric (Wang *et al.* 1999 and Whittaker *et al*. 1996).

Adopting the improved analytical techniques to screen the components of petroleum products to determine their concentrations depends on the total peak area between a fixed retention times for the marker compounds, this gives the TPH concentration. In the bioremediation process the concentration is usually calculated as a percentage of degradation (Bento *et al.* 2005). Using chemical analysis to measure hydrocarbons in soil would be as a result of degradation that is expected to occur during the bioremediation process.

However, in an attempt to accelerate the degradation of the bioremediation process nutrients could be added to augment the process and bio-waste could be added (Abioye *et al*. 2012). Using waste in this manner will direct the flow of both hazardous and biodegradable wastes from landfill to a sustainable reuse and offer the opportunity for biodegradable waste not to be considered as residue, but as a raw material for other processes. The next section will focus on waste management in the U.K. and the use of biodegradable waste in bioremediation with the emphasis being on BSG.

2.8 Waste management

The current combination of production, consumption and waste disposal in the world is seen to be incongruent with sustainability principles. The current consumption levels could be ascribed to a significant proportion of greenhouse gas emissions emanating from the use of non-renewable resources, energy and water (WRAP, 2009). The goal of the United Kingdom government is to reduce waste, re-use, recycle and recover value from wastes, which is an added value to the vision of the living planet. These goals can be fulfilled if fewer natural resources are used and our consumption of waste materials could be increased through re-use and recycling.

The U.K. government approach of the waste hierarchy is a strategy directed to all parts of society from businesses, retailers, consumers, local authorities and the waste management industry and requires them to take responsibility for the management of waste (*URL2*) The waste strategy is meant to stimulate investment in the infrastructure for the collection, recycling and recovery of waste and to improve the market for recovered materials that would optimise value and energy recovered.

In the U.K. waste generation runs into 100 million tonnes ranging from household, commerce and industry and most of the waste ends up in a landfill where the biodegradable part generates methane a potent greenhouse gas (*URL3*).

In order to enforce the waste strategy the U..K Government has adopted the EU Landfill Directive 99/31/EC which reduces the amount of harmful and biodegradable waste going to landfill. The U.K. Landfill Regulation of 2002 is in compliance with the EU Directive and states that by 2010, 2013, 2020 there should be a reduction in the amount of waste sent to landfill of 75%, 50%, and 35% of 1995 levels respectively (EC, 1999). The Directive targeted harmful and biodegradable waste which normally results in emissions of greenhouse gases including methane and carbon dioxide.

The waste hierarchy bestows responsibility to various stakeholders in the waste management industry including the following:

1. Prevention method: Whilst local authorities cannot stop consumers or retailers from generating waste, businesses or manufacturers can build resource efficiency into the business model to produce less waste by design. However, its total prevention cannot be achievable.

2. Re-use of materials: This encourages the use of materials that would otherwise become waste, which can provide a range of social, economic, and environmental benefits (Defra, 2008). In the U.K. there are value driven organisations such as voluntary and community organisations, charities, cooperatives, and social enterprises that are involved in the process of re-use of waste. There are a 1000 third sector organisations working in the U.K. that participate in the re-use of un-used material that would have been considered waste (*URL3*).

3. Recycle/recover: This is an initiative that encourages individual, corporations and the waste management industry to reduce waste going to landfill. In the waste strategy for England and Wales 2007, there are provisions for a voluntary code of practice to set out guidelines for the provision of public access to recycling facilities. The code of practice recommends five major elements that must be addressed for recycling waste, they are:

- provide recycling opportunities along with provisions for the correct disposal of waste that cannot be recycled;

- adopt standard signage on all recycle bins;
- maintain and upkeep bins;
- re-use, recycle and/
- or compost the materials collected

Recycling now provides an avenue for the public to recycle their waste such as cans, bottles and papers. The responsibility to maintain the recycling facilities rests with the local authority, which in addition must provide a convenient recycling service for household and commercial customers. In order to achieve this, the local authority must invest in new infrastructure and provide local leadership and management for waste. If a waste is not re-used or recycled then it could be composted as an alternative to waste that cannot be physically recycled back to the system. Composting of materials could take the form of anaerobic and aerobic breakdown (Defra, 2007). Green waste composting is seen as a solution to the problem of mitigating biodegradable waste volumes and to generate income from the sales of compost materials (Probert *et al.* 2005 cited in McMahon *et al*. 2008). Compost materials have become potentially useful in a wide range of applications such as land reclamation, agriculture, top soil manufacturing, horticulture and home gardening (McMahon *et al*. 2008).

4. Energy recovery: waste generation is unavoidable but the materials should be seen as reusable resources. Waste such as feedstock, minerals, organic matter and nutrients can be used for energy recovery (U.S. EPA, 1995a). The most common source of energy recovery is through incineration and the capture of methane generated at landfill sites. Incineration has become popular in most E.U. countries due to it its energy recovery ability (Dijkraaf and Vollebergh, 2004). Incineration is the controlled burning of waste at high temperatures. The organic constituents in the waste are released as exhaust gas and the mineral matter is left as residual ash (Dijkraaf and Vollebergh, 2004). In addition, landfill gas could be used to generate electricity through the production of methane.

5. Disposal method: The EU landfill directive set thresholds that by 2010 the amount of biodegradable waste going to landfill should be cut to 75% of the amount produced in 1995; and by 2013 this should be reduced to 50% and by 2020 this

amount should be further reduced to 35% (Defra, 2005). Currently the final waste disposal method is still dominated by landfill despite regional and national regulation to curtail its popularity. It is often considered to be the worst option amongst the waste hierarchy due to its use of space, high risk of leakages to air, water and soil and that it releases less of the energy content of the waste as compared to incineration (Dijkraaf and Vollebergh, 2004).

2.9 Classification of waste

In an attempt to manage waste, it is essential to identify and where possible quantify the type of waste arising from various sources. Consequently, these classifications will differ greatly in their economic potentials, their preferred disposal method such as prevention, reduction, recycle and their final destination (Defra, 2011). In the U.K. waste are classified by the Department of Environment Food and Rural Affairs (Defra) The classifications include:

1. Agriculture waste: These wastes include pesticide containers, plastics such as silage wrap, bags and sheet, packaging waste, tyres, batteries, clinical waste, oil and old machinery. Agricultural waste includes a range of waste streams that originate from agriculture or horticulture establishments.

2. Hazardous waste: This is a waste that contains hazardous properties that may cause harm to human health or the environment. These include wastes resulting from exploration, mining, quarrying, physical and chemical treatment of minerals. Others are waste from agriculture such as horticulture, aquaculture, forestry, hunting, fishing, and food preparation and processing. In addition, waste from wood processing such as production of panel and furniture, pulp and paper, cardboard, leather, fur and textile. Most Industrial waste comes from petroleum refineries, natural gas purification, pyrolytic and treatment of coal. The list of hazardous wastes is endless but can be accessed from the framework directives list put forward by the European Union legislation.

3. Construction industry: This type of waste comes mostly from the demolition and excavation sector. There is a major waste generation from the construction industry.

4. Inert waste: Waste is considered to be inert if it does not undergo any significant physical, chemical or biological transformation. Inert waste does not dissolve burn or

otherwise chemically react. It does not biodegrade or adversely affect other matter it comes in contact with. The total leachability, pollutant contact and eco-toxicity of inert wastes are insignificant and do not pose any threat to the ecological system (WRAP, 2003).

5. Waste wood: These are wastes classified from sources in varying quantities and levels of purity. There are 10 million tonnes of these types of waste produced in the U.K. each year and the bulk of it goes to landfill (Defra, 2008). These types of waste are regarded as biodegradable and they are generated mainly from the construction and demolition sector, municipal solid waste and commercial and industrial sector.

BSG is a non-hazardous waste from the production of alcoholic and non-alcoholic beverages (WRAP, 2010). The code for BSG in the European Waste Catalogue (EWC) is 02 07 02 and it comes under waste from organic chemical processes (WRAP, 2010). In the U.K. it is sold as an animal feedstuff, as a by-product, but in excess it will go to landfill, hence in some circumstances it could considered as a waste. In other parts of the world such as Asia it goes to landfill as a waste product (Mussatto *et al.* 2006).

In the study reported here it will be used to evaluate the potential for the use of biodegradable wastes in bioremediation because it was readily available. In addition, BSG will also be used to evaluate the concept of using organic waste in the bioremediation of contaminated land including their sustainability strands. These evaluations are discussed in the next sections.

2.9.1 Brewery spent grain (BSG)

BSG is a type of industrial by-product from brewery processes all over the world in both developed and developing countries. Other by-products of the brewery process including spent hops, sludge, and yeast are all regarded as wastes (Levic *et al.* 2006). BSG is the most abundant brewery by-product, corresponding to about 85% of the total 155 by-products produced (Tang *et al.* 2009). In the process of brewing and packaging beer and spirits, such as whisky, the generation of by-products and waste products is unavoidable (Levic *et a*l. 2006). The annual world production of beer is about 1.34 billion hectolitres and it is regarded as the fifth most consumed beverage in the world after tea, carbonates, milk and coffee (Fillaudeau *et al*. 2006).

The EU are known to produce 3.4 million tonnes of BSG every year and the U.K. produces over 0.5 million tonnes of this by-product annually (Stojceska *et al.* 2008).

BSG is of a high nutritive value and contains cellulose, hemicelluloses and lignin with a high protein content, which varies according to its constituents. BSG is a heterogeneous matrix comprising of a mixture of barley grain husk, pericarp and fragments of endosperm (Forssell *et al*. 2008). It is the main by-product generated in the brewing process, where barley malt is partially liquefied and the resultant liquor (wort) is separated from the solid residues (BSG). The filtered wort is finally brewed to beer. BSG has a high fibre content (cell wall carbohydrates) and is rich in proteins and other minerals. This is due to the process of beer production when nearly all of the starch is removed and the protein with other components become more concentrated. The chemical composition of BSG is a function of barley variety, harvest time, mashing condition, the quality and type of adjuncts used in the brewery or the technology employed (Santos *et al*. 2003).

There are a number of reports on the constituents of BSG from different researchers and biotechnologists. The protein and the lignin content in BSG depends on the raw materials used and the processing method applied (Forssell *et al*. 2008). BSG contains 31% of protein, 19% of pentosans, 16% of lignin, 12% starch and b-glucans, 9% of cellulose, 9% of lipid, and 4% of ash (Prentice and Refsguard, 1978). The chemical composition of BSG reported in several literatures was tabulated as follows by Aliyu and Bala (2011):

Components (% dry weight)	Kanauchi et al.,(2005)	Russ et al. (2005)	Mussatto and Roberto (2006)	Mussatto et al, (2008)	Adeniran et al. (2008)	Khidzir et al. (2010)
Cellulose	25.4	23-25	16.8	16.8	-	-
Hemicellulose	-	30-35	28.4	28.4	-	-
lignin	11.9	7.0-8	27.8	27.8	-	-
Proteins	24	19-23	15.3	-	2.4	6.4
Ashes	2.4	4-4.5	4.6	4.6	709	2.3
Extractives	-	-	5.8	-	-	-
Others	21.8	-	-	22.4	-	-
Carbohydrates	-	-	-	-	3.3	-
Crude fibre	-	-	-	-	6.4	-
Moisture content	-	-	-	-	-	-
Lipid	10.6	-	-	-	-	2.5
Acid detergent fibre	-	-	-	-	-	23.3
Total carbon (%)	-	-	-	-	-	35.6
Total nitrogen (%)	-	-	-	-	-	1.025

Table 2.2: Chemical composition of BSG as reported in the compilation of Aliyu and Bala (2011)

Due to the chemical composition and its varied attributes BSG has been found to be useful in many fields including agriculture as animal feeds, in the food industry as value-added compounds such as xylitric and lactic acid amongst others (Aliyu and Bala, 2011) and because of its fibre rich and protein rich flour, it can be used as ingredient in baking. Formulated foods are derived by grinding and sifting of dry BSG (Chaudhary and weber 1990). It can also be used for microorganism cultivation or as a raw material for extraction of compounds such as sugar, protein, acids and antioxidants (Aliyu and Bala 2011). Apart from its usefulness in human nutrition and animal feeds it could serve as alternative in biotechnological processes and for energy production (Mussatto *et al*. 2006) and its use for the generation of biogas or direct combustion are currently being investigated (Keller-Reinspach, 1992). In

addition, BSG possesses nutrients such as nitrogen, phosphorus and potassium (N, P, K) which are limiting nutrients in hydrocarbon contaminated soil and these are important elements for soil amendment and would be critical if used for bioremediation.

2.9.2 BSG as a Bio-waste

BSG could be classified as an industrial waste according to the classification by Defra. The Environment Agency, (2010) describe bio-waste as the biodegradable components of municipal waste including organic industrial waste. Although BSG could be put to many uses, it has received little attention as a marketable commodity despite being produced in large quantities. Its disposal is often an environmental problem (Mussatto *et al*. 2006). Brewery by-products including BSG are usually disposed of to landfill where the organic carbon is converted into carbon dioxide and methane (Mussatto *et al.* 2006).

There are numerous reports on the use of biodegradable waste to augment land contamination in the field of bioremediation and it has been proved to be successful for the bioremediation of contaminated soils (see Adesodun and Mbagwu *et al*. 2008; Hickman and Reid, 2008; and Liebeg and Cutright, 1999; and Jorgenson *et al.* 2000). This technique becomes popular because the product provides nutrients for microbial growth and hence enhances the breakdown of contaminants from the soil.

The current European Union directives that divert the amount of biodegradable waste going to landfill coupled with the landfill tax have opened a new spectrum for the alternative use of biodegradable waste. This study will discuss the options available to utilise bio-waste for land remediation and consider how these fit into a sustainable ethos with particular emphasis on BSG. The next section of this literature review considers the sustainability of remediation of contaminated land and how indicators for the measurement of sustainability could be developed.

2.10 Sustainability

Sustainability has been fully defined as ‘development, which meets the needs of the present without jeopardising the ability of the future generation to meet their own needs (WCED, 1987). The concept of sustainability could be seen to be product of a national agenda because it encompasses all areas of national development. It

entails three strands – economic, environment and social (Defra, 2010). Sustainability could be viewed differently by different groups of people depending on the stakeholders involved. It is only through the understanding of the perspective of each group that all stakeholders could come to a mutual agreement on a beneficial intended goal. As such industrialised countries are becoming aware of the concept of sustainability, with a broad-based government strategy being incorporated into the economy from public to private projects. In most cases there is guidance on how public sector proposals or projects should be appraised prior to commencement of funding (Euro-Demo, 2006).

In the US for instance the strategy has been proposed in the 'National Environmental Act' of 1969 compelling projects that have large impacts on the natural environment and the rest of the society to be assessed (Moon, 2003). The US EPA developed guidelines for preparing economic and environmental analysis of all costs and benefits of private or public projects that affect the environment including social costs (Moon, 2003). In the U.K. strategy for sustainable development, guidelines have been proposed by HM treasury normally referred to as the 'Green Book'. The book is the best guide for all governmental departments and executive agencies and includes projects of all types and sizes (HM Treasury, 2011). The goal is to promote efficient resource allocation of any proposed government project and take into cognizance of the wider social implications of the proposal.

The U.K. strategy for sustainable development has an aim of providing good quality of life for present and future generations with objectives of maintaining a high and stable level of economic growth without compromising the social progress and effective protection of the environment. In other European countries, such as Sweden and Norway the valuations of environmental projects have gained wider attention and they are increasing in popularity in recent times (Moon, 2003).

In achieving the goal of sustainable development in the international scene there are a number of initiatives that have evolved at a range of levels, international, regional and nationally to protect human health and the environments. For example the United Nation Framework Convention on Climate Change (UNFCCC) and Kyoto protocol, have established a framework within which many countries, can take action to mitigate the effects of Greenhouse gas (GHG) emissions. The countries that

signed up to the Kyoto agreement have committed to a domestic and national goal by developing and promoting initiatives that will limit the volume of greenhouse gases emitted given the targets set by the Kyoto agreement.

In addressing the three strands of sustainability there is a need for efficient environmental regulations with empirical estimates of the costs and benefits of environmental standards and policies (Cropper, 2000). The next sections will focus on the application of sustainability to the remediation of contamination land and how the three strands are measured.

2.10.1 Application of sustainability to remediation of contaminated land

Scientific information in the last decade suggests that global climate change can be correlated with fossil fuel use and carbon dioxide releases into the atmosphere (Ellis and Hadley, 2009) and remediation of contaminated land are usually associated with the use of fossil fuel. As such, integrating sustainability into remediation projects has attracted attention from remediation practitioners (Owsianiak, 2012). In the U.K. there has been little work on the relative sustainability of remediation techniques based on the evaluation of their wider environmental impacts (Harbottle *et al*. 2005). The objectives of sustainable remediation is to achieve remedial goals using more efficient, sustainable strategies that maximise resources and at the same time protect air, water and land (Reddy and Adams, 2010).

However, the evaluation of all potential impacts in an integrated assessment process are hardly achieved (Pollard, *et al.* 2001). This is because there is no single tool or approach for identifying and evaluating the sustainability impacts of remediation actions, as different tools focus on different elements of sustainability (Therivel, 2004). Thus, most remediation methods entail a wide range of activities that result in environmental, social and economic impacts. In the past the most obvious impacts are cost and duration and this informs the choice of appropriate technology (Harbottle *et al.* 2005).

In the U.K. assessment of economic social and environmental impacts of remediation is undertaken by an inter-government agency under the auspices of Ministry of Environment. Policies and guidelines for assessing environmental projects are directed by the Department of Environment Food and Rural Agency

(Defra), who provide a guidance role in setting the goals and objectives in achieving sustainable remediation targets and it is the main repository of all U.K. remediation data including sustainable indicators. The incorporation of all elements of sustainability into decision making during site investigation, operation of treatment systems, monitoring of treatment processes and progress and post remediation are increasingly being promoted by the relevant governmental body. For example the Environment Agency has produced guidance on the selection of remediation methods, which allows the evaluation of the effects on soil, water, air and ecosystems as a result of remediation processes (Postle *et al*. 1999).

There are varieties of tools used to evaluate the impacts or actions of remediation methods and different tools are designed to deal with the different aspects of sustainability (Therivel, 2004). However, there is no single tool or approach for identifying and evaluating the sustainability impacts of any remediation method, rather any tool should provide decision makers with information on how to minimize any negative impacts or reject any actions with significant impacts (Thierivel, 2004). Commonly used tools in the U.K. and elsewhere are cost and benefit analysis, environmental impact assessment, cost-effective analysis, multi-criteria analysis, sustainability analysis and life cycle analysis (EA, 1999, CL:AIRE, 2007, Hiester *et al.* 2002; Moon, 2003, Owsianiak *et al*. 2012; SuRF-UK, 2010 and Therivel, 2004). The adoption of any of these techniques should recognise all the embracing concepts of the three strands of sustainability otherwise the agenda will fall short of its ambitious objective.

The implementation of any remediation tool will necessitate the collection of data covering the full range of sustainability issues. Data collection normally involves the use of indicators relevant to the remediation activities. The next section will discuss the metrics and indicators recommended to be used in the U.K. by Defra when carrying out remediation projects.

2.10.2. Measurement of sustainable indicators in the U.K

The remediation techniques adopted in the U.K. show that the remediation industry is still in its infancy in respect of measuring sustainability (Defra, 2010). CL:AIRE (Contaminated Land: Application in Real Environment), developed a remediation impact assessment tool on how to measure the sustainable elements of a project.

The tool is a spread sheet that stakeholders within the remediation industry can use to assess sustainability of different remediation projects (SuRF-UK, 2010). The parameters include pollutants, such as greenhouse gases, air, water, waste, and environmental aspects of resources and people.

There is no single universally acceptable method of quantifying the impacts of company activities on sustainability (Ellis and Hardley, 2009). But the Department of Environmental Transport and Regions (DETR) in the U.K. provide indicators that capture the link between economy and environmental performance. According to Defra, (2008) there are 22 key performance indicators for U.K. businesses to measure their environmental performance. These include:

a. Emissions to air

- Greenhouse gasses
- Acid rain, eutrophication and smog process
- Dust and particulates
- Ozone depleting substances
- Volatile organic compounds
- Metal emissions to air

b. Emissions to land

- Pesticides
- Metal emissions to land
- Acids and organic pollutants
- Waste (landfill, incinerator and recycling)
- Radioactive waste

c. Resource use

- Water use and abstraction
- Natural gas
- Oil
- Metals
- Coal
- Minerals
- Aggregate

- Forestry
- Agriculture

These are quantifiable metrics that reflect the environmental performance of the economy. For stakeholders who want to measure their environmental performance the appropriate use of these tools in alignment with recognised guidelines and frameworks will demonstrate a progress toward sustainable development.

These set of guidelines are referred to as ‘Headline’ indicators of sustainable development which increase the emphases on social implication of environmental projects. The purpose of any environmental appraisal is not only to estimate the costs and benefits but seek to refine possible options of achieving the same goal with lower costs and greater environmental and social benefits. Since sustainability is a relatively new concept in most segments of the developed world and even newer to the remediation industry (Ellis and Harley, 2009), the Department of Environment, Transport and Regions in conjunction with its inter-government agency, for many years now, have provided guidance to the public and private sectors on how environmental appraisal should be carried before funds are invested.

Recently, the U.K. sustainable remediation forum (SuRF-UK) provided a framework document (SuRF-UK, 2010) on the mechanism for stakeholders to undergo sustainability assessment using approved guidelines. These indicators do not constitute a binding guidance for remediation practitioners but are recommended for use when evaluating contaminated land and water. These indicators are described below:

No. of impacts	Environmental	Social	Economic
1	Impacts on air (including climate change	Impacts on human health & safety;	Direct economic costs & benefits
2	Impacts on soil;	Ethical &equity consideration;	Indirect economic costs and benefits;
3	Impact on water;	Impacts on neighbourhoods or region;	Employment and capital gain;
4	Impact on ecology;	Community involvement and satisfaction;	Gearing
5	Use of natural resources & generation of waste;	Compliance with policy objectives & strategies;	Life-span and project risk
6	Intrusiveness.	Uncertainty and evidence.	project flexibility

Table 2.3: Showing Categories of economic, environmental and social indicators for sustainability assessment of remediation of contaminated sites adapted from Defra 2010.

These 18 headline indicators for assessing soil and groundwater remediation were developed by the United Kingdom's Sustainable Remediation Forum (SuRF-UK) and were obtained directly from the SuRF-UK report (2010). If these indicators are optimally selected, designed and implemented remediation activities may impart greater benefits than if the contaminants had been left untreated.

Generally, land and water remediation is embarked upon to mitigate risk to human

health including potential environmental, social and economic impacts. The process of sustainable remediation necessitates an assessment of all aspects of remediation processes. The process involves using indicators and other appropriate guidelines and frameworks to measure the sustainability as recommended by Defra (2008). The indicators are meant to address a range of issues that may be relevant and provide assistance to practitioners to identify the most important factors associated with the project that needs sustainability assessment. The indicators centre on the three strands of sustainability which include:

1. The economic costs of remediation

Remediation costs have been viewed as site-specific due to the variability of geological, hydrological, chemical factors and differences in remediation targets (Defra, 2010). The economic costs of any remediation project are made up of the direct costs of executing the project, which includes the costs of carrying out a feasibility study, direct costs of labour, cost of acquiring land, cost of raw materials, excavation or refill cost, landfill tax, and maintenance costs depending on the remediation technology chosen (McEntee, 1991).

Other costs that cannot be easily quantified in remediation are the life span and project risks. The economic cost of remediation is a major driver for the re-development of sites in the U.K. in addition to the time frame for the remediation objective to be met. If the remediation objectives are not on time there could be a potential loss of investment and financial risk, especially if a loan has been obtained to finance the project. However, the choice of meeting the objective is a function of the remediation technique that is adopted. More so, costs that may be considered include issues that could affect the work such as community disturbance, contractual, environmental, procurement and technological risks, reputational damage, energy use and waste generation (Defra, 2010).

Besides costs, there may be benefits that are associated with the remediation project these include employment and capital gains of job creation. Others are skill levels before and after remediation, and opportunities for education and training including innovation and new skills. Some costs and benefits can easily be monetised because they can directly be quantified by their activities and services. Whilst other costs and benefits are difficult to assign a price to due to the difficulties of using

market metrics to quantify them, these include creating opportunities for inward investment, use of funding schemes, and ability of the project to affect other projects in the locality to enhance economic values.

2. Environmental costs

In appraising remediation projects the impacts encompass the life cycle of the project and include: emissions, energy use, costs (direct and indirect), damage to ecosystems, and emissions during and post remediation work. Measuring the environmental impacts is an opportunity to assess the footprint of clean-up activities throughout the life of the project.

Parameters included are: greenhouse gasses, air, water, waste and environmental aspects of resources and people. In assessing environmental pollution in the U.K. according to the Defra guidelines and framework, data are normally collected from institutions such as the Department for Environmental Food and Rural Affairs (Defra, 2008), Department for Energy and Climate Change (DECC) and Devolved Administration. The U.K. emission inventory is compiled by an inventory team AEA technology (NAEI, 2008).

AEA compiled the data for the National Atmospheric Emission Inventory (NAEI) and all types of atmospheric emissions from all sources (Mckinnon and Piecyk, 2009). As the NAEI derives its data sources from the regulators, emission factors are typically arrived at across range of sources that are representative of the sector. Emission maps for the whole of the U.K. are routinely produced. These emission maps are used by AEA and other organisations for a variety of government policy support work at the national scale. Emission estimates in the U.K. are calculated by applying emissions factors derived by NAEI to an appropriate activity.

3. The social costs of a remediation

This is a new phenomenon in the field of remediation which is still open to debate both in terms of what the boundary is and the measurements that should be carried out. In the SuRF-UK framework, social costs may account for social justice or equality and questions that often surface are: is the spirit of the polluter pays principle adhered to with regard to the distribution of impacts and benefits and are the effects of works disproportionate to or more beneficial towards particular groups.

The social impacts may ask questions about the time scale of the impacts, if there are issues of inter-generational equity and other ethical questions. Answers to these questions determine the boundaries of how social costs should be measured.

The social element of sustainability may take the form of community satisfaction or dissatisfaction, which cannot be measured generically, rather a qualitative assessment is required to measure community satisfaction. In assessing social costs the assessor must comply with policy objectives and strategies set by regulatory bodies, codes of conduct set out nationally or by local authorities and/or in line with industry working practices and expectations (Ellis and Hadley, 2009).

However, measuring the social elements of remediation is a difficult task to undertake depending on the impacts that are being measured. There is a lack of market to generate a price for such impacts, but the outcome for this lack of market price is not accidental, rather markets are often missing this price due to the non-excludable nature of the damage caused by the impacts. Although there are economic techniques to establish monetary values for non-market impacts, they are based on the inference of price. Money values for changes in biodiversity and direct comparison with monetary values of alternative options, such as benefits of an investment project certainly provide the bases for biodiversity policies (Nunes *et al.* 2001).

For example one way to assess the social benefits of removing toxic waste from a site is to look at the impact of the site on adjacent house values over a given period of time. House values generally are determined by the discounted value of the future rents that would be accrued over time but if the existence of toxic waste at a site causes rents to fall because the unit is less desirable then house prices close to the site will decrease. Contaminated land or hazardous waste sites have a number of local impacts, as residents will complain of odour, leachate, seepage, methane gas build up, increased traffic flows, health risks, wind-blown litter, loss of visual amenities and other environmental hazards. There is a high possibility that these impacts could influence the value of local property. Social costs of remediation could be captured and the impacts analysed in their respective nature and occurrences (Kroeger and Manalo, 2006). According to Deaton and Hoehn (2004) there is a linear relationship between property prices and distance from hazardous waste sites in the

U.S.A. However, if cleaning the site is carried out and all the environmental blights are removed the price of houses located near the waste site is expected to rise. Therefore, comparing the house value before and after the cleaning provides a means of measuring the value captured as a result of the environmental improvement.

2.10.3 Appraisal of the SuRF and Defra guidelines

In reviewing the recommendation of SuRF-UK, it is clear that some practices and objectives of the guidelines can be achieved in any given remediation project. However, it is not practical to implement all of the 18 indicators on every project in that each remediation project is site-specific as the scenario in one site may differ from other sites. As previously stated there are no standard metrics that currently exist for assessing the relative sustainability of remediation alternatives.

In reviewing the environmental indicators recommended by SuRF-UK, many items on the list can be converted into project metrics for sustainable remediation decision making, provided all stakeholders agree on the approach at the inception of the process when remedial alternatives are developed and considered. But there are difficulties in agreeing on a particular approach in the remediation industry, at the moment, because there are no regulatory standards and frameworks that stakeholders must follow.

The lack of this framework can be seen in a review conducted under the guidance of Defra (2010), in which a group of practitioners in the remediation industry were surveyed, it was found that most of them evaluated their sustainability differently using various techniques and matrices and they did not adhere to a particular approach. Their decisions to assess their remediation impacts using different tools and matrices was as a result of non-binding regulatory guidance that drives remediation practitioners to use a particular approach on a project, even when the project has the same inherent environmental, social and economic impacts.

At present the role of the regulator in the decision making process is limited and advisory and practitioners evaluating sustainability of remediation projects are likely to arrive at ambiguous or conflicting results for the same project given the same site conditions (Ellis and Hadley, 2009). For example, there is no universally accepted

way of calculating the environmental element such as carbon dioxide and other environmental footprints. This leads to the use of an array of carbon calculators creating confusion and inaccurate information (Reddy and Adam, 2010).

In addition, the framework for measuring the environmental and social impacts recommended by Defra/Surf still ignore some impacts especially when socio-economic evaluation of the clean-up costs are due to secondary emissions or community disturbance during remediation activities or the effect of existing contaminants in that locality. However, the social matrices recommended by SuRF-U.K. can comprehensively be adopted by remediation practitioners in the U.K. as social impacts are usually measured by societal preferences, due to the absence of market price. Most tools adopted for valuing social impacts are either revealed or stated in the individual behaviour. Revealed or stated methods are based on impacts on environment and human welfare (Randall, 1998).

The revealed and stated methods are popular in the U.S. and have been used to examine the value of attributes that contribute to overall housing value, these attributes include distance to hazardous waste site, environmental quality, such as air pollution, odour and other negative externalities (Kiel and Zabel, 2001 and Ihlandfeldt and Taylor, 2004). The valuation methods hinge on the economic value an individual assigns to a particular goods or services (environment and social impacts). This is commonly measured by the maximum amount of resources the individual would be willing to give up in order to obtain the goods or services in questions (Barbie, 2000). Most techniques chosen to value environmental goods are meant to retrieve consumer's preferences because the price of market goods generally exhibits a clear value an individual places on them (Kroeger and Manalo, 2006).

In the U.K. social impacts occurring during the remediation process are not often measured. Although scientific study does provide evidence of incremental noise levels at certain municipal soil waste management facilities (composting, landfill and gasification/pyrolysis) as well as noise and odour from landfill sites (Defra, 2005). There are few or no studies that attempt to value social impacts during the remediation process. This is because impacts such as community disturbance or satisfaction, impacts on neighbours or the region and impacts on human health and safety are difficult to quantify. It is not surprising that they are not measured given the

nature of the impacts. For example, there is no clear cut procedure of measuring the remediation of a site that has a causal effect on the flow of traffic on adjacent roads and this is often not taken into consideration when measuring social impacts on a site.

Whilst coverage resulting from economic and environment impacts of the remediation process is reasonable and comprehensive there are still large uncertainties in the range of values provided for social impacts. It is difficult to use a particular valuation method but some studies previously mentioned have used either revealed or stated methods, in the US, allowing monetary values of social impacts to be expressed. This will make it possible to compare, in common units the relative health and environmental effect of different remediation methods.

Therefore, there is need to define the economic, social and environmental assessments with appropriate guidelines and procedures to be followed, as this will make sustainable remediation indicators more uniform even when measured by different assessors.

2.10.4 Sensitivity analysis

This analysis focuses on how a conclusion on sustainability indicators depends on certain assumptions that are used to arrive at the conclusions. In other words the analysis tests whether the indicators used hold a wider confidence margin than accounts for differences in measuring the impacts using various techniques. It has been suggested that sensitivity analysis should be considered as an element of sustainability assessment in order to determine the variation in input data and how assumption can influence the overall outcome of an assessment (SuRF-UK, 2010). These analyses are the inherent subjective values of the assessors, as it is possible that different assessors presented with the same scenario and information may arrive at a different assessment of the optimum solution. The purpose of the analysis is to underpin the need for assessors to understand the variability of their data input as any significant changes in certain inputs may alter the outcome of the assessment.

2.10.5 Setting the boundaries of a sustainability assessment

Prior to undertaking a remediation sustainability assessment, there should be a definition and agreed boundaries for the assessment (Ellis and Hadley, 2009). If these boundaries are not pre-determined there is the possibility that different assessors can make different assumptions in respect to the limit of the assessment and this will make decisions difficult to achieve (SuRF-UK, 2010). The boundaries should state which environmental, social and economic factors are to be taken into consideration, and which benefits or impacts of the three strands should be assessed. In addition the time/duration of the benefits/impacts of remediation of contaminated land should be stated for example if it is 10years, 2 generations or over 100 years and then the benefits/impacts should be measured over the agreed timeframe. This all need to be considered at the initial stage.

2.11 Conclusions

Land contamination could be as a result of anthropogenic activities with most pollution arising from past and present industrial activities. This has led to a number of brownfield sites. Society has become attuned to the necessity of re-using brownfield land for urban development.

There are a wide range of techniques that can be used to clean-up contaminated brownfield sites including physicochemical, traditional and process based techniques such as bioremediation and thermal desorption. The techniques used are associated with activities that result in environmental, social and economic impacts; however, there is growing recognition of the importance of making clean-up of contaminated land more sustainable and thereby minimizing its economic, environmental and social impacts.

Considering the sustainability of remediation projects has attracted attention from remediation practitioners and other stakeholders and the three elements of sustainability are increasingly being taken into account when a remediation technology is being considered for use. Achieving sustainability in land remediation is a current thinking in the circle of national sustainable development in the U.K. and there is an expanding portfolio of initiatives, regulation, standards and guidelines proposed by the U.K. Government through Defra. The purpose of sustainable

development in the remediation industry is to underpin the application of sustainable principles through the development process of land regeneration and mitigate the harmful effect of land contaminants posed to the environment and human well-being.

However, many brownfield development projects do not consider the ‘time’ costs of redevelopment in terms of the sustainability of the potential remediation options. This project will attempt to evaluate both the economic and environmental costs of remediation using bioremediation with and without BSG as an example. The next chapter reports on initial laboratory based investigation to determine the practicability of this approach for diesel contaminated soils.

Chapter 3

MATERIALS and METHODS

3.1 Introduction

The aim of this chapter is to describe the methods used in the study and report on the experiments carried out to evaluate them.

This entails a series of laboratory techniques including, those developed in the laboratory for the analysis of the practical aspects of the remediation processes such as soil preparation, experimental design and maintenance, microbiological methods, analytical methods, statistical analysis and the evaluation of methods and development of techniques.

Soil preparation describes how the soil was prepared before use including the materials used in the study. The experimental design and maintenance describes how the treatments were maintained throughout the experiment including temperature, water added, pH recording and the amount of soil collected for laboratory testing.

The microbiological methods describe the type of agar used in the study to characterise the bacteria. R2A and oil agar were used in the classification of the bacteria. The aim of choosing the oil agar was to investigate if the bacteria found in the soil have the potential to degrade diesel. A similar study has been carried out by Prakash and Irfan (2011) where bacteria that used crude oil as the sole carbon source were characterised and their potentials to utilize the compounds in a mixture of hydrocarbons as a substrate were determined. The R2A agar was used to determine the bacteria counts in the treated soil. It is normally used to monitor viable bacteria growth in environmental media (Pyle, et al. 1995).

The section on analytical methods describes how the sample from the treated soil was chemically analysed using gas chromatography (GC) and describes the soil extraction process prior to chemical analysis. It also describes the type of GC and the analytical conditions used in analysing the soil.

The statistical analysis describes the types of statistical test used in the study and the reasons for the choice of the different statistical tests.

The evaluation of methods and development of techniques includes the procedure used for spiking the soil in the laboratory. This becomes necessary as the volume of diesel that is to be added to the soil is small and as a result there is a need to increase the volume of liquid, added to the soil, by diluting the diesel with another solvent. This will ensure an even distribution of the diesel throughout the soil. Acetone had been previously used in the laboratory to spike contaminated soil with hydrocarbons (Sawada et al. 2004).

As such four solvents were investigated, including water, to determine which solvent can homogenously mix the diesel in the soil. This development also involved the determination of the percentage recovery of the diesel from the spiked soil. This was carried out by adding a known amount of diesel to the soil and determining how much can be recovered. A similar technique has been used by Cam and Gagni (2001) to investigate the determination of petroleum hydrocarbons in spiked agricultural soil using commercial diesel.

In addition, different percentage recoveries were compared. This was carried out to determine if the amount of diesel recovered from the soil is proportional to the volume added. In order to make these comparisons, different percentages of diesel were added to a fixed amount of soil and the diesel compounds were extracted to determine the amount recovered. This experiment is to further validate the techniques of the study including the extraction procedures used for recovering the diesel from the soil and the GC performance.

3.1.1 Laboratory work

A series of laboratory scale experiments were carried out to determine the procedures to investigate if the addition of BSG would improve upon the degradation of diesel contaminated soil with and without the addition of BSG, which was investigated in chapter 4. The procedures include:

3.2 Soil preparation and experimental design

The soil sample used in all of the experiments was a loamy soil that was a mixture of 45% clay, 25% sand and 30% silt. This is an agricultural soil without any recent nitrogen amendments. The soil was obtained from small organic farmland in Bowes in Teesdale near Barnard Castle, U.K. The soil was initially sieved through a 2 mm

sieve to remove any stones or large particles. After the soil had been sieved it was contaminated with diesel to the appropriate concentration. Full details of the method used for the addition of the diesel can be found in section 3.7.1.

The contaminated soil was transferred into trays 30 cm in length and 21 cm wide. The trays were covered with Goretex fibre, to replicate field scale remediation and incubated at room temperature. The soil was not sterilised in order that the indigenous microbial populations could be maintained. Three replicates of each treatment were prepared. Before use the soil was subjected to chemical analysis using gas chromatography (GC) to determine if the soil was free from hydrocarbon contamination.

BSG was collected from Darwin Brewery, which is a micro-brewery with a capacity of 350 litres located at Enterprise Park in Sunderland. Prior to use the BSG was collected and stored for 3-4 days at 4^0C, in the laboratory at the University of Sunderland.10 litres of commercial diesel was purchased from a filling station in Sunderland and stored at room temperature in a closed container. The same batch of diesel was used throughout the experiments.

3.3 Experimental design and maintenance

Maintaining the experiments entailed routine mixing of the soil in the trays and spraying it with water in order to maintain the soil moisture content. A constant volume of water was added to the soil weekly throughout the experiment based on the weight of soil in each treatment types. Water was sprayed onto the replicate treatments to ensure that the soil did not become dry. The soil was aerated by turning and mixing it thoroughly in order to achieve homogeneity of any amendments added.

Three replicate samples were removed at a range of sampling times. From each treatment 23.0 g of soil was removed for both microbial analysis and for the determination of TPH.

The pH value of the soil was determined using a paste prepared by shaking 1 part of the soil with 2.5 parts of distilled water (Hendershot and Lalande, 1993), readings were taken using a Fisherbrand Hydrus 300 pH meter. Before the readings were taken, the pH meter was calibrated using standard solutions.

3.4 Microbiological methods

3.4.1 Culture media

Microbial counts were performed using R2A agar and minimal (oil) media (Gong, 2012). The formulation of the R2A and oil media is represented in section 3.4.2. At each sampling time 10 g of soil was mixed with 100 cms^3 of 0.2% by volume sterilised sodium pyrophosphate, for 30 minutes, at 20^0C using a shaker at 150 rpm (Revolution per Minute). Thereafter 1 cm^3 of the soil/pyrophosphate solution was transferred into 9 cms^3 of sterile saline solution and a ten-fold serial dilution was carried out; that is from 10^{-1} to 10^{-10} according to the technique adopted in (Moreira *et al.* 2013).

After the serial dilutions, 0.1 cms^3 of the diluted solution was removed from each sterile saline-soil/pyrophosphate mixture test tube and distributed onto petri-plates containing R2A and oil agar media.

In the oil agar plate, a disc of filter paper impregnated with diesel was placed in the lid of each plate. The diesel was used as a sole source of carbon for the organisms to utilize (Balba *et al*. 1998). Both media plates were incubated at 25^0C and were inspected after 24 hours for colony formation and thereafter every 24 hours. After 72 hours the number of colony forming units (CFU) per gram was determined (Gong, 2012). The colonies were counted using a bacterial count enumerating machine and the results were recorded.

3.4.2 Formulation of media preparations used in the study

a) R2A agar

FORMULAE	
Difco ™ minimal agar Davis Formula * per Litre	**Grams**
Yeast Extract	0.5
Proteose Peptone No. 3	0.5
Casamino Acids	0.5
Dextrose	0.5
Soluble Starch	0.5
Sodium Pyruvate	0.3
Dipotassium Phosphate	0.3
Magnesium Sulphate	0.05
Agar	15.0

Table 3.1: Composition and Formulation of R2A agar and concentration levels measured in grams used in this study (URL 4).

R2A agar was developed by Reasoner and Geldreich (*URL4*) for bacteriological plate counts of treated potable water and soil related heterotrophic micro-organisms (*URL4*). The agar helps to stimulate the growth of bacteria of differing tolerances at a lower incubation temperature and longer incubation times as it is nutritionally rich. It has also been reported to improve the recovery of such micro-organisms (*URL4*).

b). Oil agar:

FORMULAE	
Difco ™ minimal agar Davis Formula * per Litre	**Grams**
Monopotassium Phosphate	2.0
Dextrose	1.0
Ammonium Sulphate	0.5
Sodium Citrate	0.5
Dipotassium Phosphate	7.0
Magnesium Sulphate	0.1
Agar	15.0

Table 3.2: Composition and Formulation of oil agar and concentration level measured in gram used in this study (URL5).

Oil agar is most suitable for growing selected heterotrophic microorganisms (Wrenn and Venosa, 1996). In this media the carbon source is reduced unlike R2A agar and other carbon sources such as hydrocarbons (including diesel) are added externally. The micro-organisms which grow on this media would be able to breakdown the diesel for their growth because of the minimal carbon present in the media itself (Abioye et al. 2012). The agar was developed by Lederberg (*URL5*).

3.5 Analytical methods

3.5.1 Diesel extraction for gas chromatography

For each replicate sample 10 g of soil was collected according to Llado *et al.* (2012) and weighed into a bottle and mixed with 20 cms^3 of hexane. It was then placed in a shaker at 100 rpm for 24 hours at room temperature. After 24 hours the samples were filtered by vacuum funnel. Aliquots of the hexane extract were pipetted from the bottle and sealed in a gas chromatography (GC) vial. Extracts were analysed as soon as possible after extraction. If it became necessary to store the samples they were kept at 4^0C. The method for the analysis of the hexane extract is described in section of 3.5.2 below.

3.5.2 Chemical analysis of hydrocarbons

Hexane extracts were transferred to a vial of 1.5 cms^3 for GC analysis using a (GC) HP 5890, Hewlett Packard with a fused silica capillary column, equipped with a flame ionization detector (FID), Agilent, USA (US EPA, 1995b). The column used was DB-1 (30m by 0.25mm) with nitrogen as the carrier gas, at a flow rate of 2 cms^3 min^{-1}. The GC used in the study is shown in Figure 3.1

Fig. 3.1: showing Gas Chromatography (GC) HP 5890, Hewlett Packard with a fused silica capillary column, equipped with a flame ionization detector (FID) which was used in analysing the soil samples for all treatments in the study.

The temperature programme used was 65^0C min^{-1} 6^0C $min^{-1}$and 300^0C for 2 mins. This method was used throughout the experiments except experiment three (3) in which helium was used as the carrier gas but all other conditions were maintained. The extractable petroleum hydrocarbons were identified and quantified by comparison of sample height and retention time to the control samples (blank samples). Duplicates of control samples were run concurrently with the original samples at 1:5. For every 5 treatment samples run a control sample was concurrently run and any discrepancy between the control and the expected results were analysed accordingly. Gas chromatography (GC) has been used by Paiga *et al*. (2012) to determine the level of TPH in contaminated soils and the results of their study demonstrated that GC had the capacity to accurately quantify TPH in soils, irrespective of the contamination levels.

3.6. Statistical analysis used in the study

Test of significance for all results in the study

Statistical analysis used in the study was to determine the significant differences and relationships between observed values. When a significance figure of ($P<0.05$) was obtained the null hypothesis of no difference was rejected and the alternate hypothesis of existence of differences in sample values was accepted.

Levenes' test of homogeneity of variance was used to help determine the type of statistical tests needed for the analysis and the results were further compared with a Shapiro-Wilk test to test the normality of the data set. Histogram plots and Q Q plots were also prepared. Although most of the tests were found not to be significant ($p = >0.05$), some were significant ($p = <0.05$), which suggested a violation of the assumption of normality (Pallant, 2007).

In the analyses paired t-test, independent t-test and the Mann–Whitney U test, which is a non-parametric test were carried out. One way ANOVA a parametric equivalent of Mann-Whitney U test was used where appropriate. However, despite the

Shapiro –Wilk test signifying that the data was non-normal, and some of the numeric normality tests on the data appearing to give significant differences, a parametric test as well as a non-parametric test were applied. This is because the distortions of normality did not affect the tests ability to detect any differences and it has therefore been possible to compare results from parametric and non-parametric tests and be able to gain extra confidence from any conclusions drawn.

3.7. Evaluation of methods and development of techniques

3.7.1 Introduction

The purpose of these experiments was to standardize the methods to be used in the main evaluation. First a procedure was developed to test the homogeneity of the diesel in spiked soil using the same soil that would be used in future experiments. This was carried out to ensure that the diesel was evenly distributed in the soil. The second experiment was carried out to determine how much of the diesel added could be recovered and the third experiment was to determine if the % recovered from the soil is proportional to the volume of diesel added.

3.7.2 Soil spiking

As the volume of diesel that is to be added to the soil is small there is a need to increase the volume of liquid, added to the soil, by diluting the diesel with another solvent. This will ensure an even distribution of the diesel throughout the soil. The soil was left in a fume cupboard for 6 hours to allow the volatile compounds to evaporate. This first experiment was evaluated after the addition of the diesel/ solvent mixture and the most suitable solvent to be used in the study was determined..

Table 3.3 represents the concentration of diesel and solvents including water used to dilute the diesel.

Solvent Name	Soil (g)	Vol. of diesel added to the soil (cms^3)	Vol. of solvent (cms^3)	Number of Trays
Acetone	2000	200	80	3
Methanol	2000	200	80	3
Hexane	2000	200	80	3
Water	2000	200	80	3

Table 3.3: Showing grams of soil, diesel and solvents evaluated as possible diluents for diesel.

The experiment comprises of four treatments which were replicated three times. For each sample 2 kg of soil was placed in a tray. The solvents or water were added to the soil in the ratio 1:25 and the soil solvent mixture was added to the diesel. The soil diesel mixture was then mixed thoroughly. The soil was then transferred into trays and the mixture was left for 24 hours. Figure. 3.2 below shows the soil and the trays used in the laboratory.

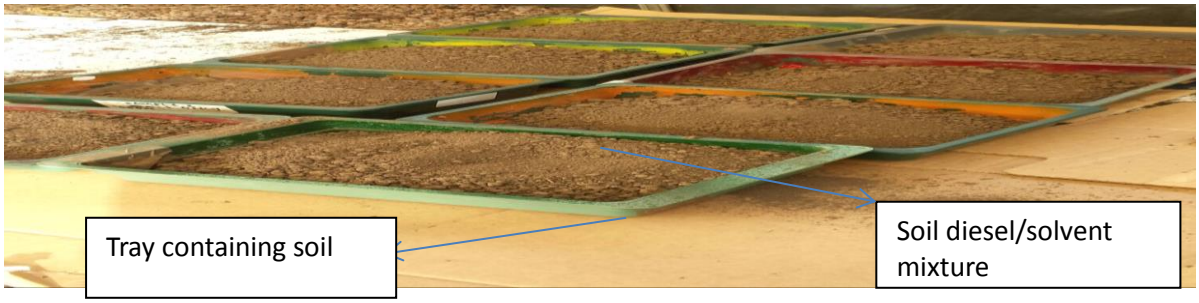

Fig. 3.2 showing replicate trays containing 2kg of soil which the diesel/solvent mixture were added.

After the addition of diesel and solvents each sample was extracted for chemical analysis as described in section 3.5.1 and 3.5.2.

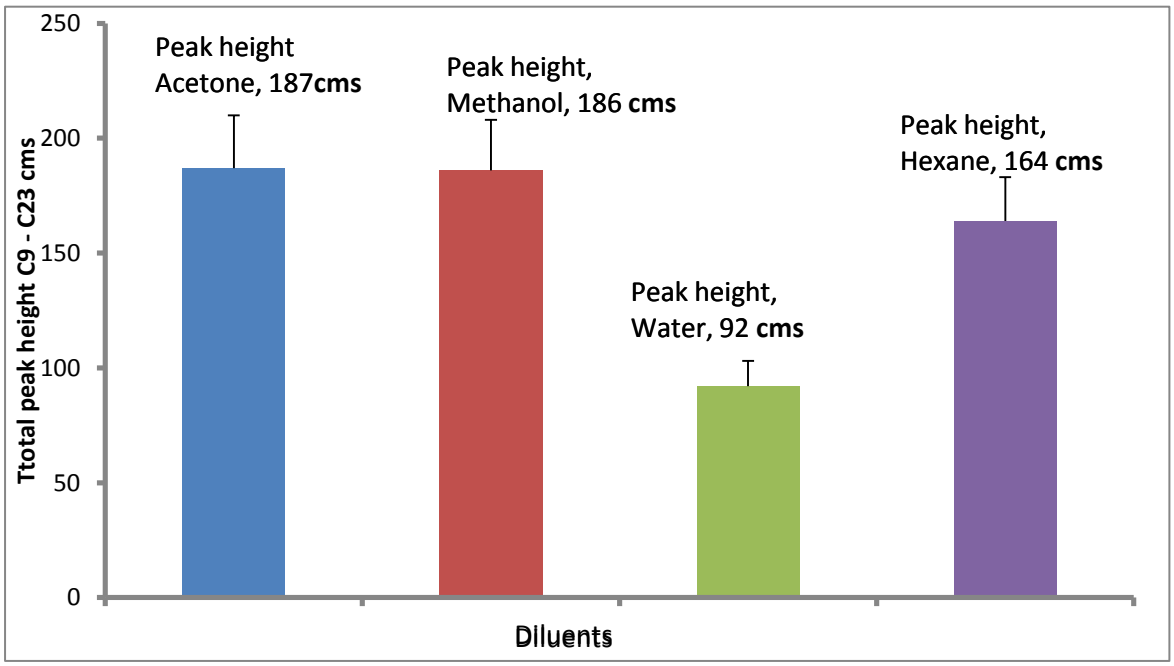

Fig 3.3: Showing total peak height as a measurement of TPH C9 – C23 recovered using the different solvents; acetone, methanol, hexane and water.

There was a statistically significant difference between treatments as determined by one-way ANOVA F (3, 48) = 5.160, p = .004. The post hoc (LSD) test revealed that recovery rates from soil contaminated with diesel mixed with acetone (M = 18.7 SD = 8.3 SE = 2.30), p = .001, diesel mixed with methanol (M = 18.6 SD = 8.0 SE = 2.2), p = .001 and diesel mixed with hexane (M = 16.4 SD = 6.9 SE 1.9), p =0.013 were

significantly greater as compared to soil contaminated with diesel mixed with water (M = 9.2 SD = 4.0 SE = 1.1). There was no statistically significant difference in height between diesel mixed with acetone and methanol p = 0.965 and between acetone and hexane p = 0.403. There was no statistically significant difference in terms of recovery between methanol and hexane p = 0.428. The post hoc (LSD) analysis is shown in table 3.4 below.

Sig/difference	**Data set of carbon ranges from C9 – C23 and their p value n=52**			
Solvent	Methanol	Water	Hexane	Acetone
Acetone	0.97	0.01	0.40	-
Methanol	-	0.01	0.43	0.97
Hexane	0.43	0.01	-	0.40
Water	0.01	-	0.01	0.01

Table 3.4: Results from one-Way ANOVA post hoc (LSD) a parametric test carried out to determine the difference in recovery for the solvents evaluated.

The results show that the average peak height, for all the constituents, was higher in acetone than the other two solvents and water. Hexane was lower than methanol and higher than water in terms of the total constituents recovered. However, in terms of the variability within the samples for each solvent and water, the results show there was less variability for the three solvents than water. The extract from the chromatogram depicting their peak height and retention time of the three solvents and water can be found in Appendix I.

Therefore, because acetone has the highest total peak height indicating greater recovery rates, it was adopted as the diluent in this study. Acetone had been previously used in the laboratory to spike contaminated soil with hydrocarbons (Sawada *et al*. 2004).

3.7.3 Percentage recovery

Recovery studies usually involve the addition of a known amount of analyte to a sample and then determining what % of the amount can be detected (Sawada *et al.* 2004). In the study here the recovery rate was tested by adding a known amount of diesel to a fixed amount of soil to determine the recovery %. In preparing the experiment 1% of diesel was added to 1 kg of soil as described in the protocol in section 3.5.1 and 3.5.2 and extracted in hexane solution. For comparison 1% of diesel was extracted (not added to soil) in hexane solution as described in section 3.5.1 and 3.5.2. Both samples were replicated. The % recovery was determined by comparing the percentage difference between TPH extracted from the soil diesel mixture and that measured when the diesel was added directly to haxane.

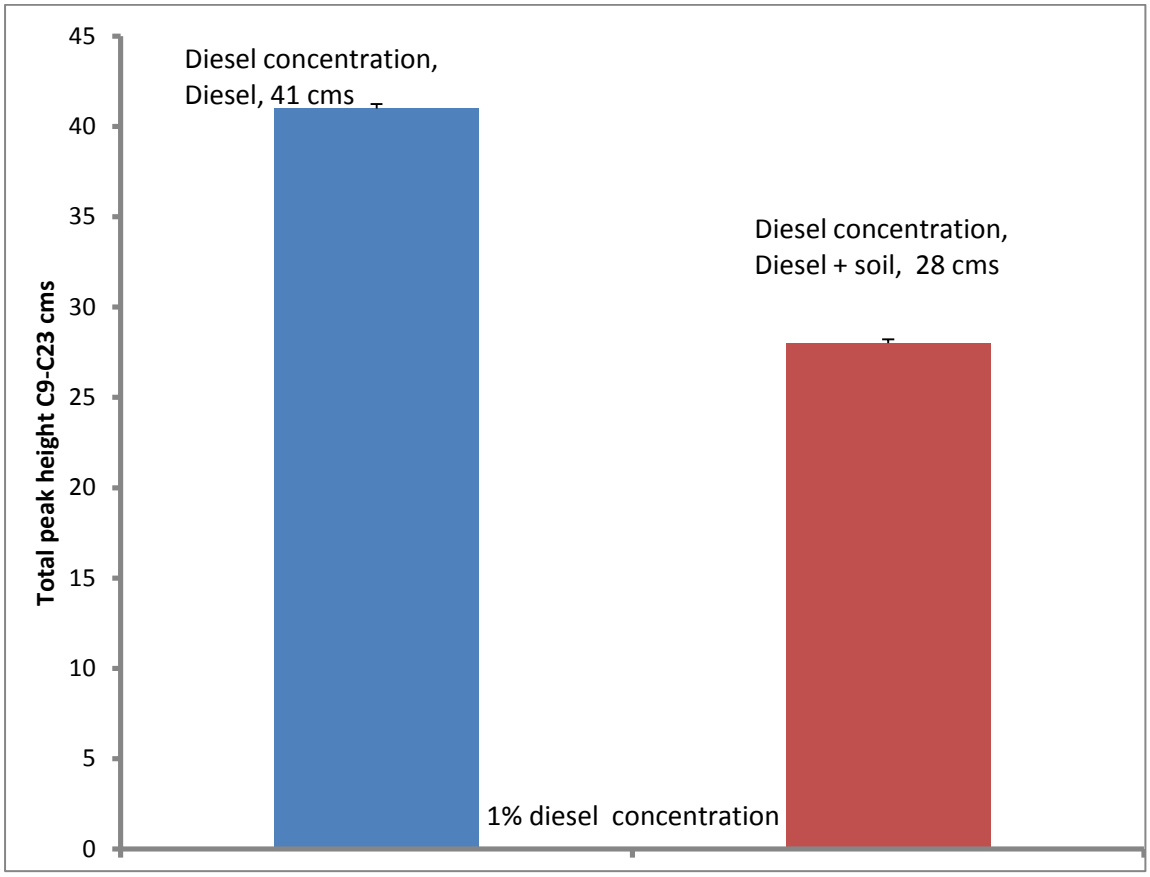

Fig: 3.4: Recovery % of diesel when added to 1 kg soil under investigation, peak height is based on carbons C9 – C23.

Figure 3.4 shows the average peak height of the treatment with 1% diesel without soil and treatment with 1% diesel added to soil. The treatment with diesel only has a total peak height of 41 cm, treatment with diesel and soil has a 28 cm level of peak

height. This is equivalent to 68% of the original diesel recovered from the soil. The statistical test to show the significant difference is shown below.

An independent sample t-test indicated that the total peak height of diesel that was added directly to the hexane was significantly higher (M = 219 SD = 128) than the total peak height of diesel extracted from the soil (M = 80 SD = 43), t(20), 4.22, p = .001, d = 1.18. Despite the differences between the samples the results showed that the recovery % of diesel from the soil was 68% based upon a comparison of the peak heights in figure 3.2. The remaining 32% could be attributed to volatilization and evaporation of the diesel due to its volatile components.

3.7.4 Representation of the effect of concentration on % recovery

This is an analytical procedure to test the relationship between multiples samples. This can be carried out by measuring known properties of different compounds in a sample and the results are directly compared to the concentration (amount) of the analyte in the sample (Harmonised, 2005). The aim here is to compare the relationship between the different % recoveries to determine if the amount of diesel added to the soil is proportional to the diesel recovered.

This experiment is to determine if the % recovery, as measured by peak height, is proportional to the volume of diesel added. Diesel was added to 1 kg of soil at a concentration of 0.5%, 1.5%, 2.5%, 3.5% and 4.5% (v/w) according to the spiking procedures described in the protocol in section 3.7.1.Each of the diesel concentrations was in triplicate as shown Figure 3.5 below:

Fig.3.5: showing replicate trays containing 1 kg of soil which the diesel/solvent mixture were added at 1%, 1.5%, 2.5%, 3.5% and 4.5% concentration level.

After 24 hours the diesel was recovered by extracting in hexane as described in

section 3.5.1 and 3.5.2. The results obtained can be found in Figure 3.6 below.

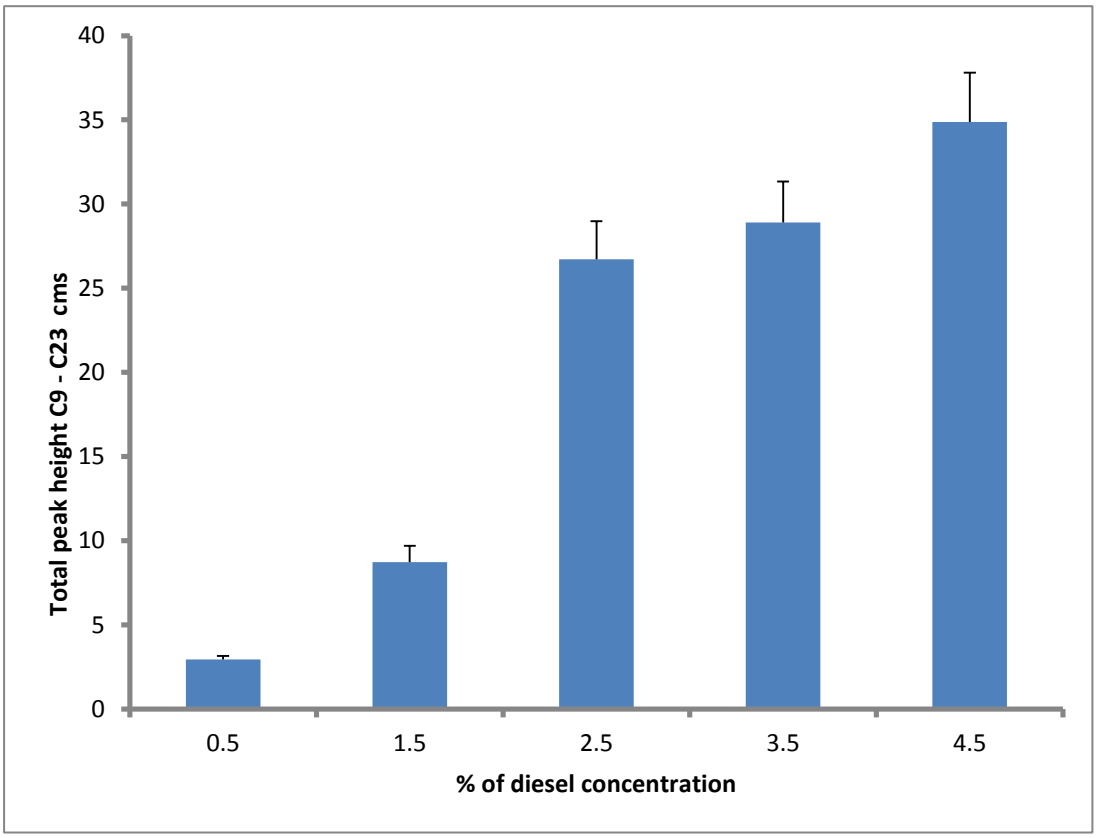

Fig 3.6: Showing different diesel concentrations added to a fixed amount of soil.Peak height is based on carbons C9 – C23

Figure 3.6 shows the total peak height of diesel concentration, with the carbon range of C9 – C23, when 0.5%, 1.5%, 2.5%, 3.5% and 4.5% of diesel were added to 1 kg of soil. The results for this experiment indicate that the highest peak height was obtained for the highest concentration of diesel (4.5%). The lowest peak height was obtained from the lowest concentration of diesel. There was a positive correlation between the amount of diesel added to the soil and total peak height achieved r = 0.839, n = 65, p = 001. The results indicate that as the concentration of diesel added to the soil increased the total peak height also increased, indicating that the amount of diesel recovered from the soil was proportional to the amount added.

3.8: Determination of water holding capacity (WHC)

Water is an essential component in cell processes as it aids the transportation of nutrients to the microbes. It also, serves as a medium for microbial growth and

regulates soil temperature. The composition of a clay-soil and its organic matter complexes govern microbial activities in the soil matrix (Paul & Clark, 1998). This also, determines the availability of water infiltration, oxygen tension and nutrient movement as required by microbial communities.

Calculating the water holding capacity (WHC) would enable the determination of the volume of water that is needed to be added to the soil to enhance microbial growth and maintain the soil structure. However, in the experiment here a consistent volume of water was added to the soil to maintain the experiment and in all cases this was 85 cms^3 for treatment without BSG and 176 cms^3 for treatment with BSG in 1000 g of soil, which is less than the field water holding capacity. The amount of water added to the soil showed that the soil was moist but not waterlogged. The water holding capacity for soil and BSG are calculated in Appendix 11 according to the description in Grace *et al.* (2006) which is the water holding capacity expected to be used in the field.

3.9 Conclusions

This chapter evaluates a range of techniques including those developed in the laboratory for the analysis of the practical aspects of the bioremediation processes. The methods covered were soil preparation and experimental design, experimental maintenance, microbiological methods, analytical methods to be adopted, statistical analysis and development of techniques.

The chapter also covered the use of various solvents to determine which solvent will be used to increase the volume of diesel when spiking the soil in order to achieve an even distribution of diesel in the soil. Acetone was adopted as the diluent to be used throughout the experiment. The results from the chapter also showed that 68% of diesel can be recovered from the soil when 1% of diesel was added to 1 kg of soil. The method further showed that the amount of diesel added to the soil was proportional to the amount recovered validating the extraction procedures and the GC performance to be used in the main experiment (Chapter 4).

Therefore, the next chapter will evaluate if the use of BSG would improve upon the bioremediation of soil contaminated with diesel fuel using the same methods.

Chapter 4

LABORATORY SCALE EVALUATION OF BIOREMEDIATION PLUS BSG

4.1. Introduction

The aim of this chapter is to investigate the use of BSG to improve the bioremediation of soil spiked with diesel in the laboratory. Two experiments were carried out - experiment (a) and (b). Experiment (a) investigated bioremediation, including the addition of BSG to augment the bioremediation processes. The experiment further studied the effects of the addition of a bacterial consortium. Experiment (b) was a repeated bioremediation process to determine the consistency of using the BSG, no bacterial consortium was added. The next sections will cover the procedures used in carrying out the experiment, which includes a review of the bioremediation techniques, methods, monitoring and maintaining the experiments, microbial analysis, results of the experiment, discussions and conclusions.

4.1.1 Review of bioremediation techniques

The prerequisite for effective bioremediation of contaminated soil is the presence of microorganisms able to degrade the contaminants (Suni and Romantschuk, 2004). Several laboratory and field tests have demonstrated the effectiveness of bioremediation in the clean-up of soil contaminated with hydrocarbons either using indigenous microorganisms or by the inoculation of exogenous microbial consortia (selected bacteria) (Benyahia *et al.* 2005, Lee *et al.* 2011 and Ameh *et al*. 2012).

A number of studies have focussed on the addition of organic wastes to improve upon the bioremediation processes, these include the laboratory scale study conducted by Cho *et al.* (1997) in which various nutrients such as Hyponex (compost for soil amendment) and bark manure, baked diatomite, microporous glass and coconut charcoal were used as a basic nutrient for microorganisms using Kuwait oil contaminated soil. The purpose of the study was to accelerate the biodegradation of hydrocarbons during 43 weeks incubation. The result shows that 15-33% of the contaminated oil was decomposed and amongst the materials tested coconut charcoal was seen to enhance the biodegradation of the hydrocarbons most effectively.

A similar laboratory scale study was conducted at the Institute of Biological Sciences, University of Malaya, Malaysia by Dadrasnia and Agamuthu (2010) to investigate the biodegradation of diesel fuel in soil contaminated with 10% (w/w) diesel fuel amended with 10% tea leaf, soy cake and potato skin for a period of 90 days (3 months). It was found in the study that 76% biodegradation of the oil was recorded at day 84 with the treatment amended with soya cake. Whilst only 27% of the oil was degraded in the control treatment. Potato skin and tea leaf recorded 64% and 53% biodegradation respectively.

The chapter reported here will focus on laboratory studies and seek to answer the first objective of the study, does the addition of BSG improve upon the bacterial breakdown of hydrocarbons and is the technique feasible? Therefore two bioremediation experiments were carried out which include experiment (a) and (b), the methods adopted are described in the next section. Experiment (a) was to investigate bioremediation, including the addition of BSG to augment the bioremediation processes, and the experiment was further studied with the addition of a bacterial consortium. Whilst experiment (b) was a repeated bioremediation experiment to determine the consistency of using the BSG no bacterial consortium was added. Experiment (b) was carried out to further optimise the process by studying the addition of BSG using different concentrations of diesel. The description of the materials and experimental design employed in this chapter can be found in section 3.2 to 3.8 in Chapter 3

4.2 Methods

Experiment (a)

The aim of this experiment was to investigate the remediation of diesel contaminated soil using bioaugmentation and biostimulation in the laboratory. The composition of the experiment consists of five treatment types as indicated in Table 4.1 below.

Treatment Reference	% of diesel in the soil	Grams of soil + diesel	Consortium added (cms^3)	BSG grams added (10%)	Number of replicate trays
1	-	1000	-	-	3
2	0.5%	1000	-	-	3
3	0.5%	1000	-	100	3
4	0.5%	1000	200	100	3
5	0.5%	1000	200	-	3

Table 4.1: Composition of the different treatments incubated in the laboratory including soil only treatment, soil plus diesel (5,000 mg kg^{-1} soil), soil plus diesel (5,000 mg kg^{-1}soil) plus consortium, soil plus diesel (5,000 mg kg^{-1}soil) plus consortium plus BSG and soil plus diesel (5,000 mg kg^{-1}soil) plus BSG

From Table 4.1 treatment (1) contains 1 kg of soil only and has no diesel or any BSG. In treatment (2) 1 kg of soil was spiked with 0.5% diesel (v/w). The composition of treatment (3) was made up of 1 kg of soil spiked by 0.5% diesel (v/w) with the addition of 100 g of BSG. In treatment (4) 0.5% diesel (v/w) and 100 g of BSG was added together with a microbial consortia at the rate of 20 cms^3 of the mature culture to 1000 g of soil. Treatment (5) contains soil with 0.5% diesel (v/w) in addition to 100 g of BSG and 20 cms^3 of the mature culture to 1000 g of soil. The consortia in treatment 4 and 5 was sprayed onto the soil and then mixed evenly. The bacteria used in the study are *Achromobacter clevlandii and Stearothermophillus rhizophil*a. The strains were supplied by Pattanathu Rahman from Teesside University and had previously been characterised as hydrocarbon degrading microorganisms isolated from contaminated river sediment. The layout of the soil used is shown below in Fig. 4.1.

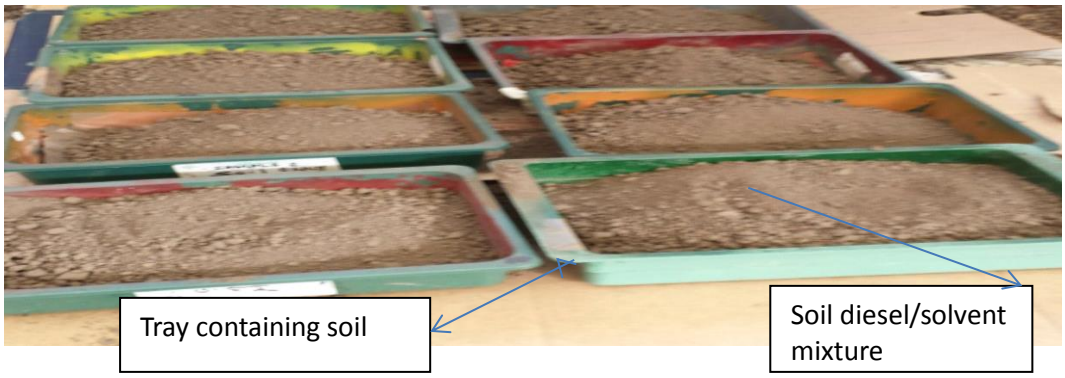

Fig. 4.1 showing replicate trays containing 1 kg of soil to which the diesel/solvent mixture was added in the laboratory.

Diesel and BSG were added to the soil treatments as described above according to the method detailed in section 3.2 and 3.7.1 Once prepared the trays were covered with Goretex fibre to prevent evaporation of the diesel and incubated at room temperature 18^0C to 25^0C for the period of the experiment. All of the experiments were set up in triplicate.

4.2.1 Monitoring and maintaining the experiment

Maintaining and monitoring the experiment involved routine mixing of the soil and the addition of water as described in section 3.3. Replicate samples were removed from each treatment for microbiological enumeration and for TPH analysis. The same protocol was applied for all treatments.

4.2.2 Microbial analysis

Quantifying the activity and number of aerobic microorganisms is an important step in evaluating the bioremediation processes. In the study reported here microbial counts were performed using R2A agar and oil agar as described in section 3.4.1.

4.3 Results of Experiment (a):

The findings from this study are presented in the following order.

4.3.1 TPH analysis

4.3.2 Statistical analysis

4.3.3 Microbial analysis

4.3.4 pH and temperature analysis

4.3.1 TPH Analysis

Results for the breakdown of TPH during the experiment can be found in Figure 4.2.

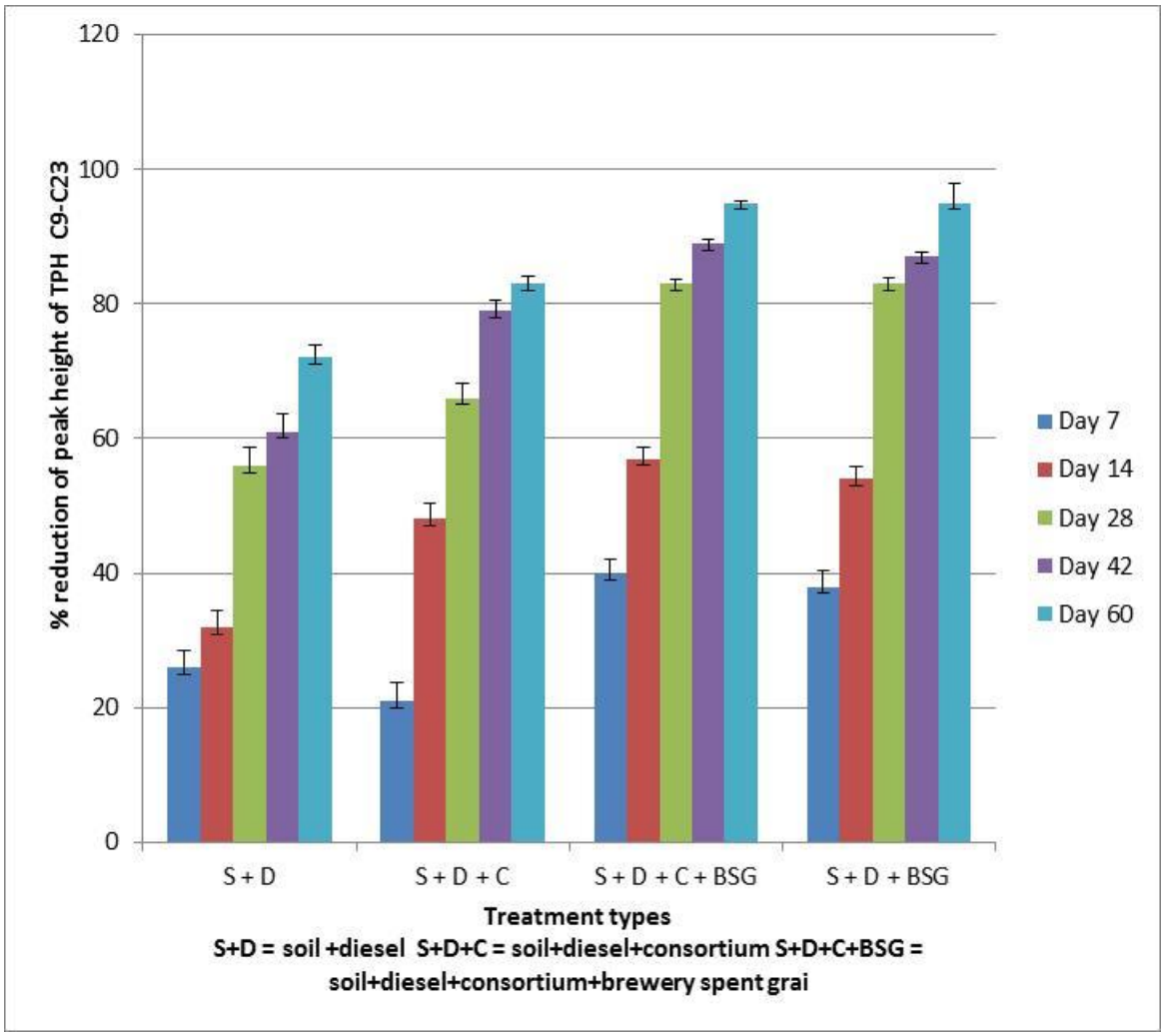

Fig.4.2: Percentage reduction of TPH on day 7, 14, 28, 42, and 60 after treatment of soil with diesel, soil plus diesel plus consortium, soil plus diesel plus consortium plus BSG and soil plus diesel plus consortium plus BSG.

Fig 4.2 illustrates the peak height reduction of the different treatment types from day 1 to 60. These were calculated as the percentage reduction of the height using the initial height as the baseline and measured against subsequent reduction in height over time. There was a 32% reduction of TPH at day 14 for the treatment with soil plus diesel and the addition of consortium brought about a 41% reduction in TPH.

The introduction of BSG to the consortium resulted in a 56% reduction and the treatment with soil plus diesel plus BSG also recorded a 56% reduction. At day 28 the treatment with soil plus diesel shown a reduction of 55% and the treatment with soil plus diesel plus consortium has a reduction of 66% but the addition of BSG brought about an 82% reduction in the total peak height. Whilst the treatment with soil plus diesel plus BSG recorded an 83% reduction at day 28.

At day 42 the treatment with soil plus diesel has a reduction of 61% whilst the addition of consortium led to a further reduction of 77% and the addition of BSG resulted in an 89% reduction. The treatment with soil plus diesel plus BSG has a TPH reduction of 87% at day 42.

Finally, at day 60 the treatment of soil plus diesel has a total reduction of 72% and treatment with consortium has an 83% reduction. The addition of BSG to the consortium led to a further reduction of 95% and treatment with soil plus diesel plus BSG also recorded a total reduction of 95% at day 60 respectively.

4.3.2 Statistical results of TPH

The results from Fig. 4.2 above indicated that there were differences in the TPH reduction in the various treatment types. Analysis of variance using one-way ANOVA and a post hoc test to show the differences between the treatment types for day 1, 14, 28, 42, and 60 are presented below.

	Data set and p value for day 1 to 60 n= 52				
Treatment types	Soil + Diesel day 1	Soil + Diesel for day 14	Soil + Diesel day 28	Soil + Diesel day 42	Soil + Diesel day 60
S + D + C	0.67	0.44	0.43	0.08	0.15
S + D + C +BSG	0.53	0.13	0.01	0.01	0.01
Soil + D + BSG	0.79	0.25	0.01	0.01	0.01
Treatment types	Soil + D + C for day 1	Soil + D + C for day 14	Soil + D + C for day 28	Soil + D + C for day 42	Soil + D + C for day 60
S + D + C +BSG	0.29	0.02	0.01	0.14	0.01
Soil + D + BSG	0.49	0.06	0.07	0.23	0.01
Treatment types	Soil + D + C + BSG for day 1 P value	Soil + D + C + BSG for day 14 P value	Soil + D + C + BSG for day 28 P value	Soil + D + C + BSG for day 42 P value	Soil + D + C + BSG for day 60 P value
Soil + D + BSG	0.72	0.70	0.92	0.78	0.98
Where = S+D+C soil+diesel+consortium S+D+BSG soil+diesel+BSG S+D+C+BSG soil+diesel+consortium+BSG					

Table 4.2: *Results from one-Way ANOVA post hoc (LSD) a parametric test carried out on treatment with soil plus diesel, soil plus diesel plus consortium, soil plus diesel plus consortium plus BSG and soil plus diesel plus BSG*.

Day1

There was no statistically significant difference between groups for the mean of various treatment types as determined by one-way ANOVA F (3, 52) = 0.395, p = 0.757. The post hoc (LSD) test revealed that there was no significant difference between the total peak height for treatment with soil plus diesel and soil plus diesel plus consortium p = 0.670 and treatment with soil plus diesel plus consortium plus BSG p = 0.534 and treatment with soil plus diesel plus BSG p = 0.790.

The treatment with soil plus diesel plus consortium did not statistically differ from treatment with soil plus diesel plus consortium plus BSG p = 0.296 and treatment with soil plus diesel plus BSG p = 0.490. There was no significant difference between the treatment with soil plus diesel plus consortium plus BSG and treatment with soil plus diesel plus BSG p = 0.721.

Day 14

There was no statistically significant difference between group means for the various treatment types as determined by one-way ANOVA F (3, 52) = 2.190, p = 0.096. Post hoc (LSD) test revealed that there was no statistically significant difference between the total peak height for treatment with soil plus diesel and treatment with soil plus diesel plus consortium p = 0.443 and treatment with soil plus diesel plus consortium plus BSG p = 0.133 and treatment with soil plus diesel plus BSG p = 0.257.

There was no statistically significant difference in total peak height for treatment with soil plus diesel plus consortium and treatment with soil plus diesel plus BSG p = 0.060. There was no significant difference between the treatment with soil plus diesel plus consortium plus BSG and treatment with soil plus diesel plus BSG p = 0.704. However, the treatment with soil plus diesel plus consortium plus BSG was significantly lower in total peak height (M = 130 SD 63 SE 1.6) when compared to the treatment of soil plus diesel plus consortium (M = 199 SD 87 SE 2.3), p = 0.025.

Day 28

There was a statistically significant difference between the group mean as determined by one-way ANOVA F (3, 52) = 3.547, p = 0.021. Post hoc (LSD) test revealed that the treatment with soil plus diesel was significantly higher in total peak

height (M = 106 SD 102 SE 2.7), compared to the treatment of soil plus diesel plus consortium plus BSG (M = 37 SD 28 SE 0.7), p = 0.010 and the treatment with soil plus diesel plus BSG (M = 39 SD 32 SE 0.8), p = 0.013.

However, there was no statistically significant difference between soil plus diesel plus consortium and treatment with soil plus diesel (p = 0.433) and treatment with soil plus diesel plus consortium plus BSG (p = 0.064) and treatment with soil plus diesel plus BSG (p = 0.079). There was also no statistically significant difference in total peak height between the treatments with soil plus diesel plus consortium plus BSG and treatment with soil plus BSG p = 0.920.

Day 42

There was a statistically significant difference between group means as determined by one-way ANOVA F (3, 52) = 4.263, p = 0.009. Post hoc (LSD) test revealed that treatment with soil plus diesel was significantly higher in total peak height (M = 94 SD 96 SE 2.5) compared to treatments with soil plus diesel plus consortium plus BSG (M = 24 SD 20 SE 0.5), p = 0.002 and treatment with soil plus diesel plus BSG (M = 30 SD 26 SE 0.6), p = 0.005.

However, there was no statistically significant difference between soil plus diesel plus consortium compared to treatment with soil plus diesel p = 0.089 and treatment with soil plus diesel plus consortium plus BSG p = 0.147 and treatment with soil plus diesel plus BSG p = 0.237. There was no statistically significant difference in treatment with soil plus diesel plus consortium plus BSG when compared to treatment with soil plus diesel plus BSG p = 0.783.

Day 60

There was a statistically significant difference between group mean as determined by one-way ANOVA F (3, 52) = 5.301, p = 0.003. Post hoc (LSD) test revealed that treatment with soil plus diesel was significantly higher in total peak height (M = 66 SD 72 SE 1.9), compared to treatment with soil plus diesel plus consortium plus BSG (M = 11 SD 11 SE 0.3), p = 0.002 and treatment with soil plus diesel plus BSG (M = 11 SD 11 SE 0.3), p = 0.001.

However, there was no significant difference in total peak height between the

treatment with soil plus diesel plus consortium and treatment with soil plus diesel p = 0.155 and treatment with soil diesel plus BSG p =0.061 and treatment with soil plus diesel plus consortium plus BSG p = 0.064. And there was no significant difference between treatment with soil plus diesel plus consortium plus BSG and treatment with soil plus diesel plus BSG p = 0.984.

The above results (Table 4.2) indicate that there were differences in the TPH reduction amongst treatment types. The one-way ANOVA test carried out on the data showed these differences between the treatment types from day 1 to 60 as shown Table 4.2 in addition of the summary of the post hoc test and their p values.

4.3.3 Microbial analysis

The microbial populations of the various soil treatments were enumerated based on the two agar media R2A and oil agar. Microbial counts were performed on two substrates R2A Agar and oil media with hydrocarbon as a food source, full details can be found in Chapter 3. A summary of the mean values for both agar plates are represented in Table 4.3 (log transformed).

Treatment types	Agar plates	Sampling days (log transformed) counts			
		Day1	Day 14	Day 28	Day 60
Soil only	**Oil**	0	0	0	0
	R2A	7.53	6.73	7.11	6.88
Soil + diesel	**Oil**	0	0	6.30	0
	R2A	8.00	8.35	8.11	7.84
Soil + diesel + consortium	**Oil**	0	5.89	0	0
	R2A	7.89	8.46	7.85	7.52
Soil + diesel + consortium + BSG	**Oil**	6.56	6.72	7.04	5.00
	R2A	8.64	8.51	8.25	8.41
Soil + diesel + BSG	**Oil**	6.63	6.77	7.31	0
	R2A	7.97	8.73	8.70	8.54
	BSG = Brewery spent grain.				

Table 4.3: Summary of CFU Enumeration of soil samples for R2A and oil agar plates for Day 1, 14, 28 and 60 represented in count/Log.

Table 4.3 shows the results for microbial populations of the five treatment types including the control soil. The results show the relationship between R2A and oil agar in each treatment from Day 1 to 60. The oil agar which can be termed the hydrocarbon utilizing bacteria (HUB) showed zero growth for control soil whilst R2A agar ranged from 6.73 to 7.53 from Day 1 to 60. The counts of R2A agar for the

treatments with soil and diesel ranged from 7.84 to 8.35 whilst oil agar counts were only recorded on Day 28. Counts of hydrocarbon degrading bacteria (HUB) in the treatment with soil plus diesel plus consortium showed a count of 5.89 at Day 14 only and the corresponding R2A agar ranged from 7.52 to 8.46 from Day 1 to 60. The counts of hydrocarbon utilizing bacteria in soil plus diesel plus consortium plus BSG ranged from 5.00 to 7.04 and the R2A agar ranged from 8.25 to 8.64 from day 1 to 60 respectively. Whilst that of the treatment with soil plus diesel plus BSG had counts on R2A ranging from 7.97 to 9.73 and the oil agar ranged from 6.63 to 7.31 from Day 1 to 60 respectively.

Consequently, the counts on contaminated soil were higher than the control soil, which does not have any BSG or consortium in both R2A and oil agar. Additionally, the counts on the treatment with BSG were higher than treatment with no BSG. Treatments with BSG recorded higher counts on both R2A and oil agar than the treatment with soil and diesel and the consortium.

4.3.3.1 Statistical results of microbial counts

The results from Table 4.4 below indicate that there were differences in microbial populations between the different soil treatments. Analysis of variance using one-way ANOVA and a post hoc test to show the differences between the treatment types for day 1, 14, 28, 42 and 60 were compared with the control soil and the results are presented in Table 4.4.

	Data set for p value for day 1 to 60 n=52			
Treatment types	Soil only for day 1	Soil only for day 14	Soil only for day 28	Soil only for day 60
So + D	0.31	0.01	0.09	0.01
S + D + C	0.64	0.01	0.07	0.13
S + D + C + BSG	0.01	0.01	0.15	0.01
S + D + BSG	0.05	0.01	0.03	0.01
Treatment types	Soil + D for day 1	Soil + D for day 14	Soil + D for day 28	Soil + D for day 60
S + D + C	0.55	0.79	0.90	0.24
Soil + D + C +BSG	0.09	0.39	0.85	0.07
Soil + D+ BSG	0.57	0.88	0.42	0.02
Treatment types	Soil + D + C for day 1	Soil + D + C for day 14	Soil + D + C for day 28	Soil + D + C for day60
Soil + D + C +BSG	0.01	0.50	0.77	0.01
Soil + D + BSG	0.17	0.91	0.48	0.02
	Soil + D + C + BSG 1	Soil + D + C + BSG 14	Soil + D + C + BSG 28	Soil + D + C + BSG60
Soil + D + BSG	0.14	0.46	0.36	0.68
	Where= S+D soil+diesel S+D+C soil+diesel+consortium S+D+C+BSG soil+diesel+consortium+BSG S+D+BSG soil+diesel+BSG			

Table 4.4: Results from one-Way ANOVA of microbial counts from day 1 to 60. Post hoc (LSD) a parametric test carried out on treatment with soil only treatment (control), soil plus diesel treatment, soil plus diesel plus consortium, soil plus diesel plus consortium plus BSG and soil plus diesel plus BSG.

Day 1

There was a statistically significant difference between group mean as determined by one-way ANOVA F (4, 20) = 2.946, p = .046. Post hoc (LSD) test revealed that treatment with soil only (control) was significantly lower in microbial populations (M = 7.48 SD = 0.53 SE = 0.20) compared to treatment with soil plus diesel plus consortium plus BSG (M = 8.90 SD = 0.38 SE = 0.22), p = 0.005 and treatment with soil plus diesel plus BSG (M = 8.21 SD = 0.57 SE = 0.21), p = 0.051. However, There was no statistically significant difference between treatment with soil only (control) and treatment with soil plus diesel p = 0.312 and treatment with soil plus diesel plus consortium p = 0.640.

Day 14

There was a statistically significant difference between group mean as determined by one-way ANOVA F (4, 14) = 11.102, p = 0.001). Post hoc (LSD) test revealed that bacteria numbers for the treatment with soil only (control) was significantly different (M = 6.72 SD = 0.08 SE = 0.08) as compared to the treatment with soil plus diesel (M = 8.41 SD 0.25 SE = 0.13), p = 0.001 and treatment with soil plus diesel plus consortium plus BSG (M = 8. 70 SD 0.68 SE = 0.39), p = 0.001 and also treatment with soil plus diesel plus consortium (M = 8.49 SD 0.38 SE = 0.17) , p = 0.001.

Day 28

There was no statistically significant difference between group mean as determined by one-way ANOVA F (4, 12) = 1.677, p =0 .219). Post hoc (LSD) test revealed that treatment with soil only (Control) was not statistically different in microbial populations from treatment with soil plus diesel p = 0.095 and treatment with soil plus diesel plus consortium p = 0.079 and treatment with soil plus diesel plus consortium plus BSG p = 0.153. However, the treatment with soil only was significantly lower (M = 7.06 SD = 0.18 SE = 0.11) compared to the treatment with soil plus diesel plus BSG (M = 8.47 SD 0.42 SE = 0.42), p = 0.030.

Day 60

There was a statistically significant difference between group mean as determined by one-way ANOVA F (4, 24) = 7.490, p =0 .001. Post hoc (LSD) test revealed that the treatment with soil only was significantly lower in microbial populations (M = 7.00 SD = 0.25 SE = 0.12) compared to the treatment with soil plus diesel (M = 7.86 SD = 0.89 SE = 0.34), p = 0.014 and treatment with soil plus diesel plus consortium plus BSG (M = 8.40 SD = 0.28 SE = 0.11), p = 0.001 and treatment with soil plus diesel plus BSG (M = 8.53 SD = 0.36 SE = 0.15), p = 0.001. However, there was no statistically significant difference in microbial populations between soil only (control) and treatment with soil plus diesel plus consortium p = 0.137. Details of the ANOVA table and post hoc test are shown above in Table 4.4 above.

4.3.4 pH and temperatures analysis

Treatments types	Day 1	Day 14	Day 28	Day 42	Day 60
Soil only	6.7	6.6	6.7	6.4	6.5
Soil + Diesel	5.8	6.2	6.4	6.9	6.9
Soil + Diesel + C	5.9	6.0	6.4	6.9	6.7
Soil + Diesel + C + BSG	6.3	6.9	7.0	6.6	6.6
Soil + Diesel + BSG	6.2	6.8	7.1	6.9	6.6
Soil only = Soil (control), C = consortium, BSG = brewery spent grain.					

Table.4.5: Results of soil pH values for the duration of the experiment for soil plus diesel, soil plus diesel plus brewery waste, with and without consortium and soil plus diesel plus consortium

The soil pH changed little throughout the experiment. At the beginning of the experiment the control soil had a pH of 6.7 and at day 42 it was 6.4 the pH taken at day 60 was 6.5. The treated soil plus diesel had an initial pH of 5.8 which rose to 6.4 at day 28. This further increased to 6.9 at days 42 and 60 respectively. Similarly, the treated soil plus diesel plus consortium had an initial pH of 5.9 which rose to 6.4 at day 28 increasing to 6.9 at day 42 but dropping to 6.7 at day 60.

The treated soil plus diesel plus consortium plus BSG had an initial pH of 6.3 which rose to 7.0 at day 28 and dropped to 6.6 at days 42 and 60 respectively. In a similar pattern the treated soil plus diesel plus BSG also had an initial pH of 6.2 which rose to 7.1 at day 28 but began to drop at days 42 and 60 to 6.9 and 6.6 respectively.

The average experimental temperatures recorded also changed throughout the duration of the experiment ranging from 18^0C at day 1, to 22^0C at day 42 increasing to 25^0C at day 60. The temperature was not control and could be regarded as warm throughout the duration of the experiment.

4.4 Methods

Experiment (b)

This study was carried out to determine the biodegradation of diesel in soil to which 1% (v/w) of diesel had been added with/without the addition of 10% BSG. The aim of this experiment was to further investigate the use of BSG in the bioremediation process. In order to achieve this two treatments were investigated - treatment with BSG and treatment without BSG. The amount of diesel used in each tray in this experiment was *10,000 mg* kg^{-1}*soil* and experiment (a) was *5,000 mg* kg^{-1}*soil.* The percentage of diesel added to the soil in experiment (b) was 1% compared to 0.5% in experiment (a). The methods and materials used are described in the next section.

4.4.1 Method of experiment (b)

The materials used in the study are described in section 3.2.1, 3.2.2 and 3.2.3. Table 4.6 shows the composition of the treatments and how they were applied in the study.

Treatment types	% of diesel in the soil	Grams of soil + diesel	BSG (10%) added in grams	Number of trays
1	-	2000	-	3
2	1%	2000	-	3
3	1%	2000	200	3
4	-	2000	200	3

Table 4.6: Composition of the different treatments incubated in the laboratory including soil only treatment (control), soil plus diesel (10,000 mg kg^{-}1 soil), soil plus diesel (10,000 mg kg^{-}1) plus 200 g brewery spent grain and soil plus 200 g brewery spent grain (control)

From table 4.6 Treatment (1) contains 2 kg of soil only, which is the control sample without BSG and treatment (2) contains 2 kg of soil which was contaminated with 1% diesel (v/w). The composition of treatment (3) was the addition of 1% diesel to 2 kg of soil plus 200 g BSG (w/w). Treatment (4) contains 2 kg of soil and 200 g BSG. The diesel and BSG were added to the soil treatments according to the method detailed in section 3.7.2. A photograph of the soil and trays is shown Fig.4.3.

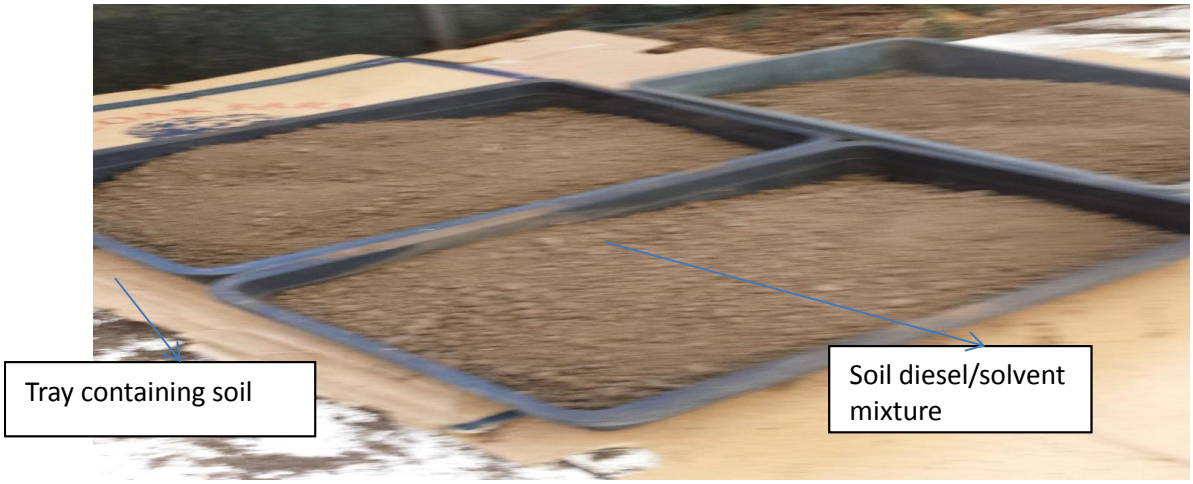

Fig. 4.3: Replicate trays containing 2 kg of soil to which the diesel/solvent were added in the laboratory.

Once prepared the trays were covered with Goretex fibre to prevent evaporation of the diesel. The average temperature ranging from 22^0C to 28^0C for the period of the experiment. All the treatments were set up in triplicate.

4.4.2 Monitoring and maintaining the experiment

In preparing the experiment the same protocol used in section 3.2 was adopted and applied. Maintaining the experiment involved routine mixing of the soil with the addition of water as described in section 3.3.

Replicate samples were removed from each treatment at 1, 14, 28, 42 and 60 days and tested for microbial activity and for TPH.

4.5 Results of Experiment (b)

The findings from this study are presented in the following order:

4.5.1 TPH analysis

4.5.2 Statistical analysis

4.5.3 Microbial analysis

4.5.4 pH and temperature analysis

4.5.1 TPH analysis

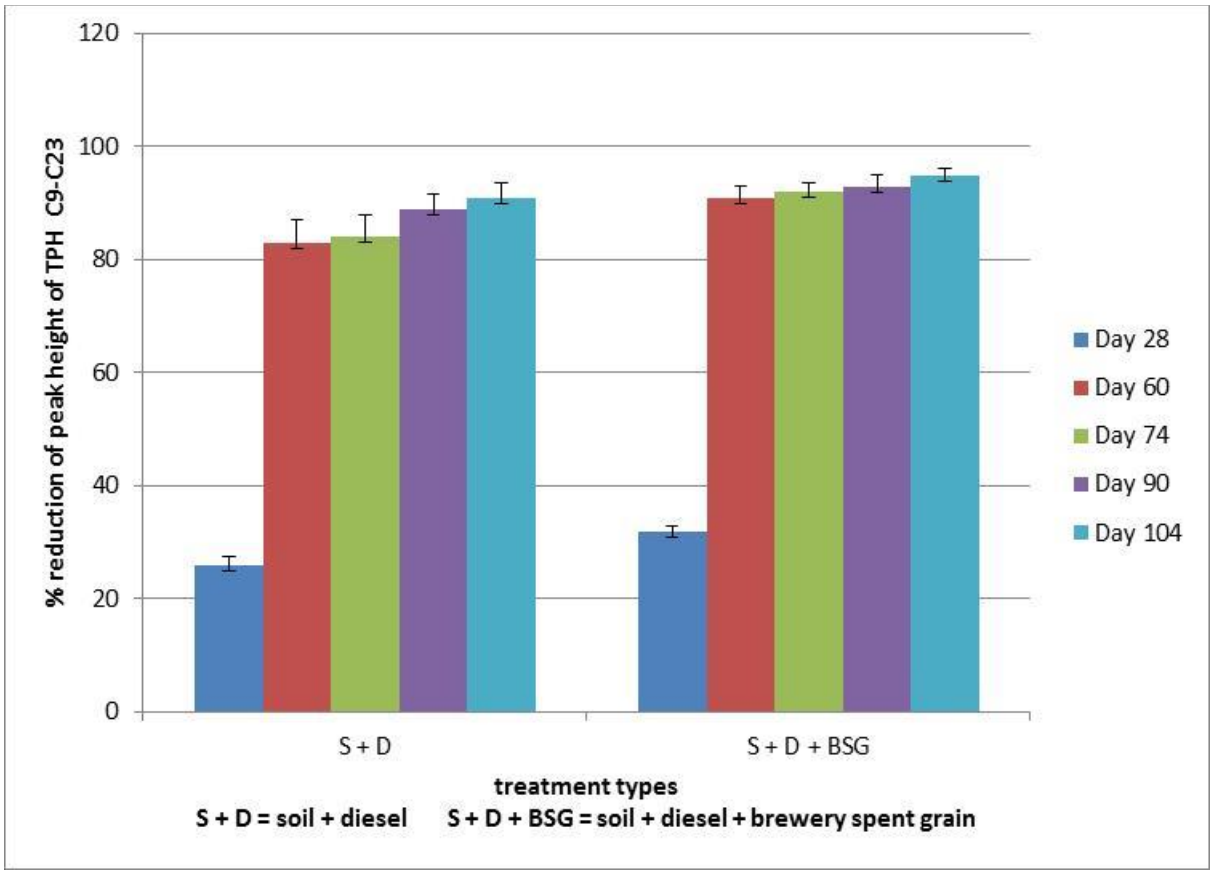

Fig.4.4: Summary of percentage reduction of TPH on day 28, 60, 74, 90, and 104 after treatment of soil with diesel and diesel plus brewery spent grain.

Fig. 4.4 above shows the results of the percentage reduction of TPH over time for each treatment. There was a 26% reduction at day 28 in the treatment with soil plus diesel but the addition of BSG resulted in a 31% TPH reduction. In the treatment of soil plus diesel there was a reduction of 83% at day 60 the addition of BSG led to a further reduction of the TPH to 91%.

At day 74 there was a reduction of 84% in the treatment with soil plus diesel the addition of BSG brought about a 92% reduction. The TPH reduction increased to 90% at day 90 and 91% at day 104 and the addition of BSG resulted in a 93% and 95% reduction respectively. The extracts from the chromatogram are shown in Figures 4.5 and 4.6 for the two treatment types.

of window 38: Current Chromatogram(s)

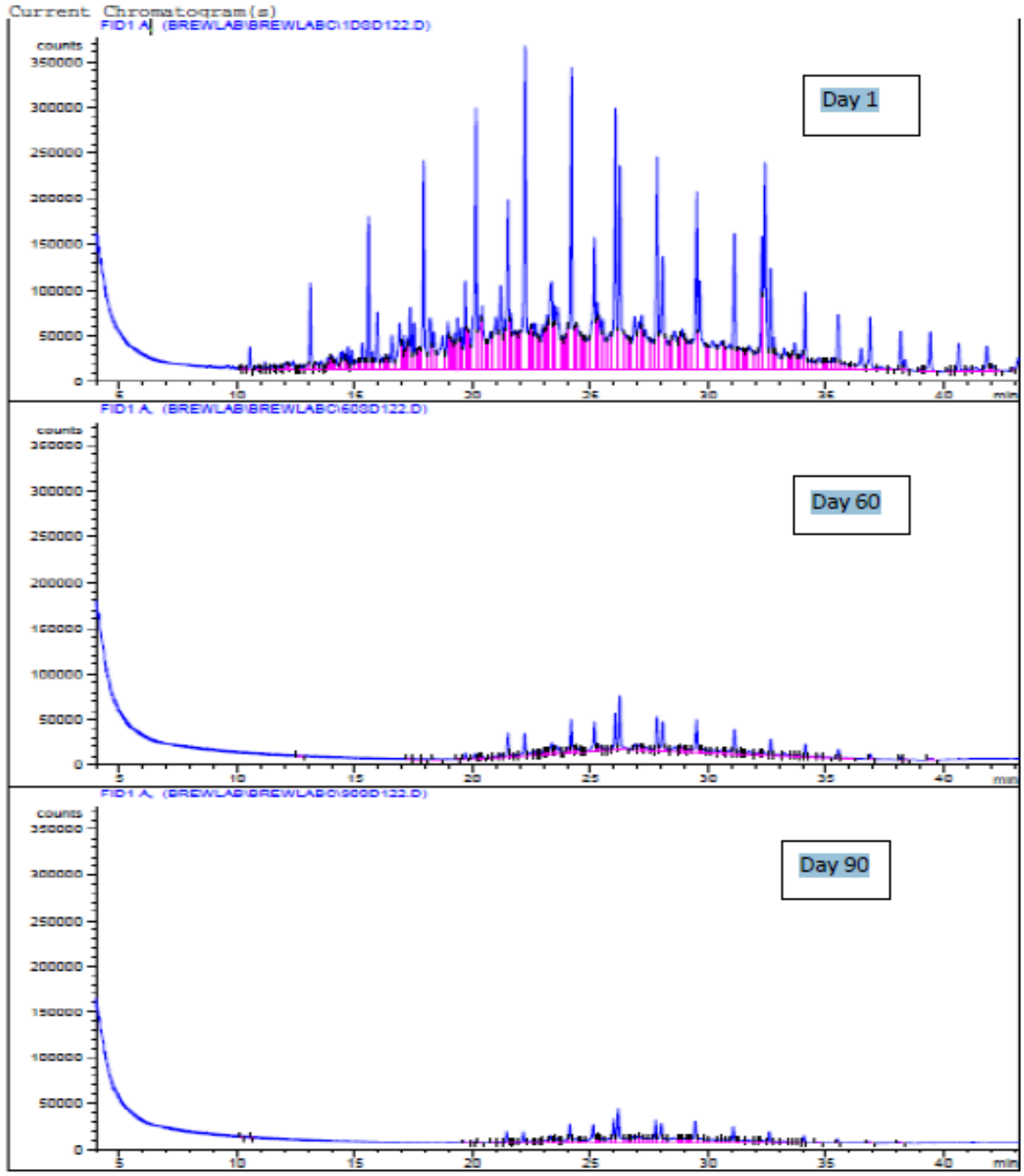

Fig 4.5: Extract from chromatogram for day 1, day 60 and day 90 for soil plus diesel showing the degradation of the diesel over time.

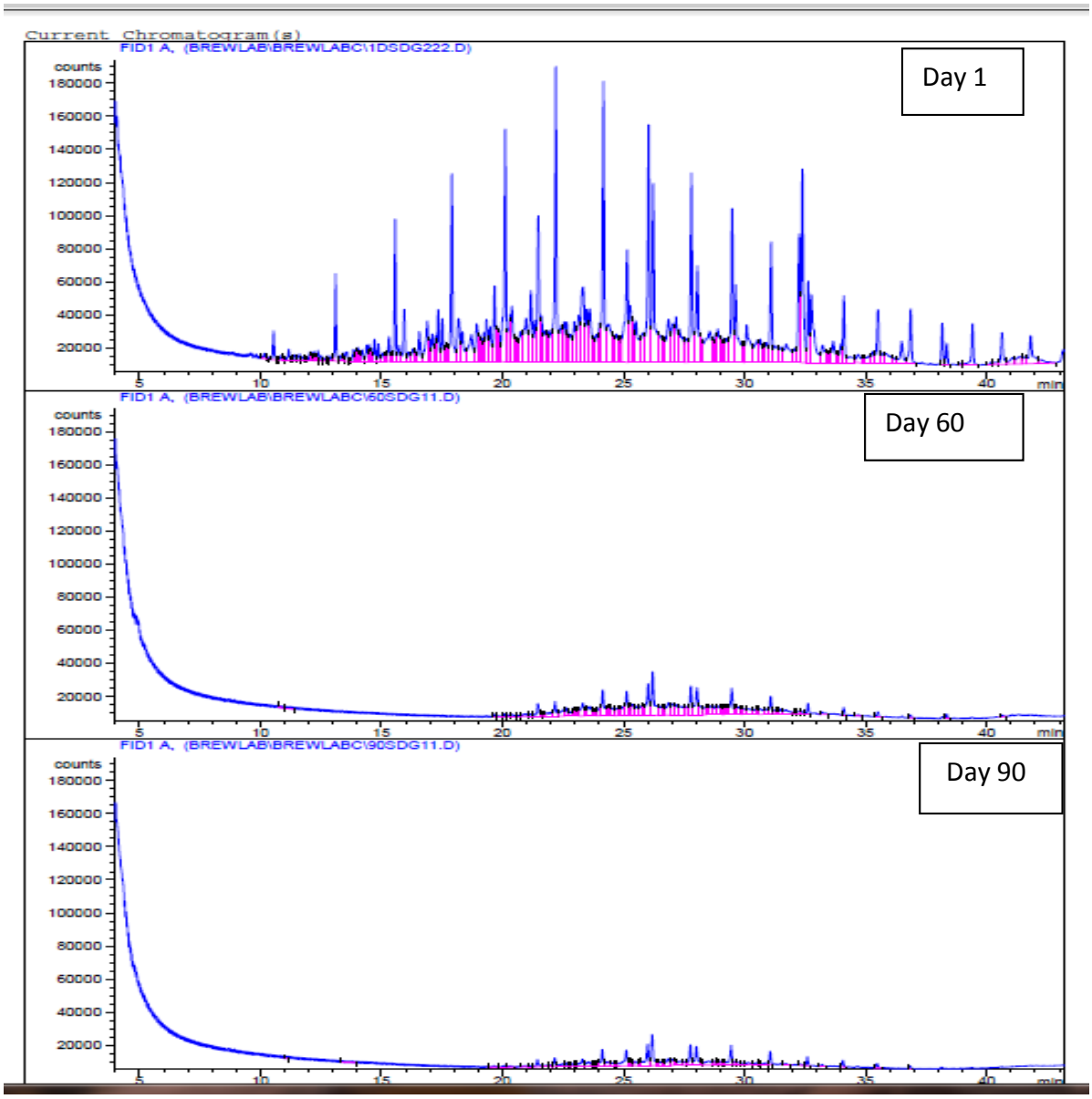

Fig 4.6: Extract from the chromatogram for day 1, day 60 and day 90 for soil plus diesel plus BSG showing degradation of the diesel over time.

Figures 4.5 and 4.6 show an extract from the chromatogram obtained from the GC for days 1 to 90. During the bioremediation process many microbes are known to use various degradation pathways for different petroleum products. In addition, most microorganisms that are known to mineralise PAH's under aerobic conditions have used similar metabolic pathway (Zhang et al. 2006). The hydrocarbons used in the study reported here was diesel with carbon molecules ranges from C9 – C23. Diesel of such nature is known to have approximatly 64% of aliphatic hydrocarbons, 1-2% of Olefinic (alkene) hydrocarbons and 35% of aromatic hydrocarbons (*URL6*). But

hydrocarbons have a different degree of susceptibility to microbial attack. The order of such decreasing susceptibility are n-alkanes > branched alkanes > aromatic of low molecule weight > cyclic alkanes (Perry, 1984).

Figures 4.5 and 4.6 show the reduction patterns of the diesel compounds with the high molecular weights expected to take a longer time to degrade. The light fractions are expected to degrade within the early stage of bioremediation (Bento, et al. 2005). In the study reported here it was observed in the GC monitoring that there was an increase in the number of low molecular weights hydrocarbons within 28 days of the experiment suggesting that the higher molecular weights were broken down, resulting in an increased number of lower molecular weights hydrocarbons. The increase in the number was qualitatively observed to be greater in soil treated with BSG than soil without BSG. This study characterised the hydrocarbon degrading bacteria responsible for degrading diesel contaminated soil using oil agar and measured the total TPH using the peak height, each of the hydrocarbon constituents were not identified. As such further studies are needed to determine if the increase in low molecular weights hydrocarbons was as a result of the addition of the BSG used to augment the bioremediation process.

The highest percentage of reduction in peak height was observed within 28 days of the experiment part of this may be attributed to volatilisation and microbial attack on the diesel compounds as reported in (Bento, et al. 2005). More so, from the results of the soil microbiological enumeration presented in the study, it may be inferred that the high percentage of diesel-degrading microorganisms indicates that there are more heterotrophic microorganisms in the soil treated with BSG than soil without BSG suggesting the population of oil degrading microorganisms influenced the degradation process. According to Salminen (2004) in order for the products of the tri-carboxylic acid TCA cycle (amino acids, protein, nucleotides and nucleic acids) to be synthesised, a nitrogen, sulphur and phosphorous source is required, in the study reported here BSG was added as a nutrient in the bioremediation process. The end processes of respiration are CO_2, water and the cell biomass (Salminen, (2004).

The reduction pattern for both treatment types has previously been presented as percentages. At day 104 the treatment with soil plus diesel resulted in 91% degradation and the introduction of BSG brought about a further reduction to 95%.

The statistical analysis carried out on the original data showed that there was a statistically significant difference between the treatment with soil plus diesel and treatment with soil plus diesel plus BSG $p = 0.05$.

4.5.2 Statistical results for TPH

The results from Table 4.7 indicate that there were differences in the TPH reduction in the various treatment types. Independent t-test (parametric) and Mann-Whitney U statistical tests were conducted on the data to show the differences between the treatment types for day 1, 28, 60, 74, 90 and 104. The reason for conducting the two tests was for the results to be compared and to gain extra confidence from the conclusions that could be drawn.

Statistical Test	Data set Day 1	Data set for Day 28	Data set for Day 60	Data set Day 74	Data set Day 90	Data set Day 104
Mann-Whitney U test (Non-parametric)	0.41	0.16	0.01	0.01	0.78	0.01
Independent t-test(parametric)	0.41	0.13	0.01	0.01	0.06	0.01
Sample Size	n= 36	n= 36	n=36	n=36	n=36	n=36
	Where * = significant difference					

Table 4.7:Results of TPH from Mann Whitney non-parametric test and a parametric independent t-test, carried out on soil plus diesel treatment data and soil plus diesel plus BSG data on day 1, 28, 60, 74, 90 and 104.

Independent t-test (parametric)

Day 1

An independent-sample t-test indicated that there was no significant difference between the total peak height for the treatment of soil plus diesel (M = 157, SD = 83) compared to the treatment with soil plus diesel plus BSG (M = 135, SD = 78), t(34), 0.817, p = 0.419.

Day 28

An independent-sample t-test indicated that there was no significant difference between the total peak height for the treatment of soil plus diesel (M = 119, SD = 60) compared to the treatment with soil plus diesel plus BSG (M = 92, SD = 42), t (34) 1.528, p = 0.136.

Day 60

An independent-sample t-test indicated that the total peak height was statistically higher in the treatment with soil plus diesel (M = 27, SD = 18) compared to the treatment with soil plus diesel plus BSG (M = 12, SD = 8), t (24) 3.129, p = 0.005.

Day 74

An independent-sample t-test indicated that the total peak height was significantly higher in the treatment with soil plus diesel (M = 24, SD = 16) compared to the treatment with soil plus diesel BSG (M = 11, SD = 7), t (23) 3.272, p = 0.003.

Day 90

An independent-sample t-test indicated that there was no statistically significant difference between the total peak height for the treatment with soil plus diesel (M = 15, SD = 11) compared to the treatment with soil plus diesel plus BSG (M = 9, SD = 8), t (34) 1.943, p = 0.060.

Day 104

An independent-sample t-test indicated that the total peak height was significantly

higher in the treatment with soil plus diesel (M = 14, SD = 11) compared to the treatment with soil plus diesel plus BSG (M = 6, SD = 5), t (24) 2.628, p =0.015. Detailed results of the independent t-test can be found in table 4.6 above.

Mann-Whitney U test (non-parametric)

A Mann-Whitney U test was conducted for all of the data across each day and between the two treatment types. It revealed that :

Day 1

There was no statistically significant difference between the total peak height for treatment with soil plus diesel (Mdn = 150, n = 36) compared to treatment with soil plus diesel plus BSG (Mdn =135, n = 36), U = .136, z = 0.14, p = 0.411.

Day 28

There was no statistical significant difference between the total peak height for treatment with soil plus diesel (Mdn = 120, n = 36) compared to treatment with soil plus diesel plus BSG (Mdn =93, n = 36), U = .118, z = 0.232, p = 0.164

Day 60

A Mann-Whitney U test indicated that the treatment with soil plus diesel was significantly higher in total peak height (Mdn = 28, n = 36) compared to the treatment with soil plus diesel plus BSG (Mdn = 13, n = 36), U = .79, z = 0.44, p = 0.009.

Day 74

A Mann-Whitney U test indicated that the treatment with soil plus diesel was significantly higher in total peak height (Mdn = 26, n = 36) compared to treatment with soil plus diesel plus BSG (Mdn = 11, n = 36), U = .80, z = 0.43, p = 0.009.

Day 90

There was no statistically significant difference between the total peak height for treatment with soil plus diesel (Mdn = 18, n = 36) compared to treatment with soil plus diesel plus BSG (Mdn =8, n = 36), U = .107, z = 0.29, p = 0.078.

Day 104

A Mann-Whitney U test indicated that the treatment with soil plus diesel was significantly higher in total peak height (Mdn = 13, n = 36) compared to treatment with soil plus diesel plus BSG (Mdn = 5, n = 36), U = .94, z = 0.36, p = .031. Detailed results of the Mann-Whitney U test can be found in table 4.7 above.

4.5.3 Microbial analysis

4.5.3.1 CFU Enumeration

The microbial populations of the various soil samples were enumerated based on the two Agar plates (R2A and oil) over 90 days (Day 1, 14, 24, 56, 70 and 90). The R2A agar is a general representation of the microbial populations and the oil agar should indicate those bacteria capable of using diesel as a substrate. The results of R2A and Oil agar are reported in Table 4.7 below in their respective log form.

Treatment	Agar plates	Bacteria counts at sampling days					
		Day1	Day 14	Day 28	Day 60	Day 74	Day 90
Soil only	**Oil**	0	2.00	0	0	0	0
	R2A	7.40	7.58	7.15	8.10	6.87	7.05
Soil + diesel	**Oil**	2.00	2.74	3.18	3.28	2.95	2.00
	R2A	7.59	7.65	7.91	8.03	7.29	7.22
Soil + diesel + BSG	**Oil**	3.34	4.09	4.73	4.67	4.00	3.18
	R2A	7.77	8.40	8.56	8.00	7.31	7.23
	BSG = brewery spent grain Oil/R2A = Count/Log						

Table 4.8: Summary of CFU enumeration of soil samples for R2A and oil agar plates for day 1, 14, 28, 60, 74 and 90 evaluated in count/log.

The results from Table 4.8 showed that there were microbial counts on R2A agar in all the treatments from day 1 to day 90. The counts recorded in the treatment with soil only (control) ranges from 6.77 to 7.56 from day 1 to day 90. The counts recorded in the treatment with soil plus diesel ranged from 7.36 to 8.18 and for the treatment with soil plus diesel plus BSG counts were from 7.29 to 8.56 from day 1 to 90.

On the other hand the results from oil agar recorded from day 1 to day 90 varied amongst the treatment types as indicated in Table 4.9. For soil only treatment the microbial counts were recorded in day 14. For the treatment with soil plus diesel the microbial counts were recorded from day 1 to 90 and ranged from 2.00 to 2.39 whilst the count recorded for the treatment with soil plus diesel plus BSG from day 1 to day 90 was 3.17 to 4.63 respectively.

4.5.3.2 Statistical analysis of microbial populations

The results from Table 4.9 indicate that there were differences in microbial populations between the different soil treatments. Analysis of variance using one-way ANOVA and post hoc test to show the differences between the treatment types for day 1, 14, 28, 42, 60, 74 and 90 and compared with the control soil are presented below in Table 4.9.

Treatment types	**Data set from day 1 to 90 and their P value**					
Soil only	Soil day 1	Soil day 14	Soil day 28	Soil day 60	Soil day 74	Soil day 90
S + D	0.631	0.10	0.01	0.19	0.12	0.26
S + D + BSG	0.27	0.01	0.01	0.33	0.51	0.84
	Soil + diesel day 1	Soil + diesel day 14	Soil + diesel day 28	Soil + diesel day 60	Soil + diesel day 74	Soil + diesel day 90
Soil + D + BSG	0.58	0.28	0.07	0.64	0.33	0.36
	Where S+D soil + diesel S+D+BSG soil + diesel + BSG S+D+BSG soil +diesel +BSG					

Table 4.9: Results of Microbial counts from one-Way ANOVA post hoc (LSD) a parametric test carried out on treatment with soil only treatment (control), soil plus diesel treatment and soil plus diesel plus BSG.

Day 1

There was no statistically significant difference between group means as determined by one-way ANOVA F(2,16) = 0.651, p = 0.535. Post hoc (LSD) test revealed that the microbial population was not significantly higher in the treatment with soil only as compared to the treatment with soil plus diesel p = 0.631 and treatment with soil plus diesel plus p = 0.272. There were no significant differences in microbial populations between the treatment with soil plus diesel plus BSG and the treatment with soil plus diesel p = 0.586.

Day 14

There was a statistically significant difference between group mean as determined by one-way ANOVA F(2,15) = 4.140, p = 0.037. Post hoc (LSD) test revealed that the microbial populations in the treatment with soil only (control) was significantly lower (M= 7.47 SD = 0.51 SE = 0.21) as compared to the treatment with soil plus diesel plus BSG (M = 8.31 SD = 0.61 SE = 0.25), p = 0.012. However, there was no statistically significant difference between the treatment with soil plus diesel, treatment with soil only p = 0.103 and treatment with soil plus diesel plus BSG p = 0.281.

Day 28

There was a statistically significant difference between the group mean as determined by one-way ANOVA F (2, 14) = 9.029, p = 0.003. Post hoc (LSD) test revealed that the microbial populations in the treatment with soil only (control) was significantly lower (M = 7.24 SD = 0.25 SE = 0.11) compared to treatment with soil plus diesel (M = 8.01 SD = 0.60 SE = 0.21), p = 0.013 and treatment with soil plus diesel plus BSG (M = 8.57 SD = 0.33 SE = 0.16), p = 0.001. However, there was no significant difference between the treatment with soil plus diesel plus BSG and the treatment with soil plus diesel p = 0.071.

Day 60

There was no statistically significant difference between group means as determined by one-way ANOVA F (2,13) = 1.033, p = 0.384. Post hoc (LSD) test revealed that

microbial populations in the treatment with soil only was not significantly higher as compared to treatment with soil plus diesel p = 0.333 and treatment with soil plus diesel plus BSG p = 0.194. There was also no statistically significant difference between the treatment with soil plus diesel plus BSG and treatment with soil plus diesel p = 0.646.

Day 74

There was no statistically significant difference between group means as determined by one-way ANOVA F (2, 9) = 1.454, p =0.284. Post hoc (LSD) test revealed that the microbial populations for the treatment with soil only was not significantly higher when compared to treatment with soil plus diesel p = 0.122 and treatment with soil plus diesel plus BSG p = 0.511. There was also no statistical difference between the treatment with soil plus diesel plus BSG and treatment with soil plus diesel p = 0.330.

Day 90

There was no statistically significant difference between group mean as determined by one-way ANOVA F (2, 13) = 0.782, p = 0.478. Post hoc (LSD) test revealed that treatment with soil only did not differ statistically in microbial populations as compared to treatment with soil plus diesel p = 0.267 and treatment with soil plus diesel plus BSG p = 0.840. There was also no statistically significant difference in microbial populations between treatment with soil plus diesel plus BSG and treatment with soil plus diesel p = 0.362.

4.5.4 pH and temperatures analysis

Treatments types	Day 1	Day 14	Day 28	Day 42	Day 60	Day 90
Soil only	6.0	6.2	6.1	6.3	6.8	6.8
Soil + D	5.3	5.4	5.2	5.8	5.7	6.1
Soil + D + BSG	5.2	5.7	5.8	6.0	6.3	6.5
Soil only = (control soil). D = diesel. BSG = brewery spent grain.						

Table 4.10: Results for soil pH values for the duration of the experiment for soil plus diesel, soil plus diesel plus BSG and for soil only.

The soil pH from Table 4.10 changes little throughout the experiment amongst the different treatment types. At the beginning of the experiment the treatment with soil only (control) had an initial pH value of 6.0 in day 1 with little changes in day 14 and 28. The pH values rose to 6.8 in day 60 and 90 respectively. In contrast to the control soil the treated soil plus diesel had an initial pH value of 5.3 with also little changes on day 14 and 28 but the pH value went up from day 42, 60 and 90. Similarly the treated soil plus diesel plus BSG had an initial value of 5.2 at day 1 after that there was a progressive increase. At day 14 the pH value was 5.7 and at day 28 it was 5.8 and at day 60 and 90 the pH value was 6.3 and 6.5 respectively.

The initial temperature was 11^0C and increased to 19^0C at 14 day. The temperature recorded at day 28 was 21^0C this went up to 24^0C at day 42 and this was maintained up until day 60. The final temperature was maintained at 27^0C from day 60 to 90.

4.6 Discussion

The measurement of hydrocarbon degradation can be assessed using both chemical and biological methods (CL:AIRE, 2006 and EA, 2010b), however effective bioremediation cannot be measured using a single parameter. Several methods used to assess microbial activities have provided a meaningful method of characterising microbial communities during bioremediation. These include microbial enumeration, soil respiration and enzyme activity such as dehydrogenase, phosphate assay and proteases (Margesin, 2000). Measures such as microbial biomass and enzyme activity could be seen as a possible means of evaluating the healthy nature of the soil and the potential success of bioremediation (Dick and Tabatabai, 1992). Dadrasnia and Agamuthu (2010) used microbial enumeration to evaluate the bioremediation of diesel-contaminated soil, where organic wastes were applied to augment the bioremediation process. Jorgensen *et al.(*2000) measured microbial activity by measuring soil respiration for composting of contaminated soil in biopiles in an ex-situ technology. Here organic matter, such as bark chips was added to the contaminated soil as a bulking agent.

The committee of In Situ bioremediation has recommended strategies for evaluating progress of bioremediation (EA, 2010a), these include: documented loss of contaminants from the site, laboratory assays which should show that the microorganisms from the site have the potential to transform the contaminants, under the expected conditions at the site. One or more pieces of information should demonstrate that biodegradation potentials can actually be realised in the field (EA, 2010b). TPH could be evaluated to determine the relative concentration of petroleum in the soil. TPH may be recorded as mg/kg, ppm or as a relative percentage of the total sample (U.S DOE, 1996).

Therefore, the purpose of this section is to discuss the results obtained from the laboratory experiments on different bioremediation processes in addition to their biological activities with regard to their usefulness for monitoring and for assessing the results of the soil experimentally contaminated with diesel. Changes in diesel concentration and the accompanying changes in microbial activities with time were measured.

4.6.1 Microbial analysis

The composition of microbial populations in soils at contaminated sites can be affected by the composition of the hydrocarbons in the soil (Admon, 2001**).** Addition of diesel to soils provides a source of carbon for any micro-organisms present, but the resultant increase in carbon may lead to an imbalance of nitrogen and phosphorus, which may prove to inhibit microbial activities (Morgan and Watkinson, 1989 and Kim, 2005). Nitrogen and phosphorous can then be a limiting factor in the bioremediation of hydrocarbon contaminated soils (Yang *et al*. 2009).

In the study presented here the plate count method was used to quantify the population and activity of the soil micro-organisms and the technique used evaluated the number of microbes (R2A) and their biodegradation potentials as measured by their growth on oil agar. The results of the microbial counts, using R2A agar showed there was a higher count in the soils with diesel and soil with BSG than the control soil as indicated in both experiments. The statistical analysis showed that the control soil was significantly lower than treated soil plus diesel and treatment with BSG $p = 0.05$. Although in experiment (b) there were lower microbial counts for R2A plates in the control soil compared to the treatment with soil plus diesel and treatment with BSG but there was no significant difference between these treatments except on days 14 and 28 $p = 0.05$. However, the differences between the treatments in both experiments could be due to the inherent heterogeneity of the mixture of soil samples after the addition of BSG or the addition of consortium as indicated in Table 4.1 and Table 4.6.

The increase in microbial counts in R2A agar for soil with diesel and treatment with amendment is expected because higher amounts of electron donors (from the hydrocarbon substrate) can support larger populations of microorganisms. This coupled with the addition of BSG which may bring additional bacteria, may also explain the higher microbial counts with treatment with BSG. In determining the possibility of bioremediation in hydrocarbon contaminated soil, the U.S EPA (United States Environmental Protection Agency) suggests that there should be 10×10^3 colony forming units per gram of soil in contaminated soil (Ta – Chen, 2010). In the present study the colony forming unit per gram of soil for both experiments exceeded this threshold.

However, there are limitations to the use of plate count methods to quantify the population and activity of soil microorganism in the study. This means increased numbers of microorganisms in a particular treatment should not be assumed to indicate the presence of microbes that can degrade the contaminants present or as strong evidence of bioremediation occurring. Using R2A agar plate counts could be misleading because of the thousands of different bacterial species in one gram of soil of which less than 1% could be culturable (Rossello-Mora and Amann, 2001). Besides, R2A agar contains simple sugars and is likely to grow those micro-organisms unable to digest hydrocarbons. This means that R2A agar will only give a general representation of the microbial populations of the soil. All these factors could contribute to the varied cell counts found, in the sample, in this study.

However, the detection of microbial activities using oil media with hydrocarbons as a carbon source could be used to determine the presence of hydrocarbon degrading bacteria as reported by (Dadrasnia and Agamuthu, 2010). Hence the results obtained with this media were used to evaluate the biodegradation potentials of the bacteria in the diesel contaminated soil. These hydrocarbon degrading bacteria were comparable to the R2A agar in all the treatment types. The difference between the two media in each treatment type could be attributed to the richness in microbial activities of the treatment with BSG and a lack of sufficient nutrients in the treatment without BSG.

An analysis of the data in Table 4.3 and 4.8 shows that the growth rates of R2A and oil agar were higher in treatments with BSG compared to treatments without BSG experiments (a) and (b) respectively. In the present study the reasons attributed to higher counts of bacteria in R2A and oil agar in soil with BSG could be due to the presence of considerable quantities of nitrogen and phosphorus in the soil with BSG, which are requisite nutrients for bacteria/ biodegradative activities (Adesodun and Mbagwu, 2008).

BSG is a beverage industry by-product having food characteristics, it is used in agriculture because of its high organic matter and is known to contain significant energy resources from its organic content (Thomas and Rahman, 2006) and soil organic matter content and biomass are related to microbial activities (Garcia-Gill, 2000). The BSG may provide organic material suitable for bacterial growth and the

growth on R2A media may not be indicative of bacterial that are breaking down the diesel however, the growth found on the oil media had diesel as a source of carbon, so bacterial growth here is indicative of those that can breakdown diesel.

In addition, the results of microbial counts in the two experiments showed that microorganisms capable of using the diesel as a nutrient source were only isolated from the contaminated soil with diesel and soil treated with diesel and BSG. These organisms were rarely found in soil without hydrocarbon or treatment without amendment. In experiment (a) the presence of the microbes were lowered across the treatment types but in experiment (b) the microbes were dominant in all the treatment types except the control soil. The difference between the two experiments was due to the dilution series adopted in the study, as dilution series for experiment (a) was 10^{-4} to 10^{-6} and experiment (b) was 10^{-2} to 10^{-4} . The dilution series with 10^{-4} to 10^{-6} recorded few or no counts in some treatments as shown in the results section. Therefore, an increase in dilution series in experiment (b) resulted in a high concentration of micro-organisms with the treatment, with BSG having higher concentrations in both R2A and oil agar compared to treatment without BSG.

4.6.2 pH values and temperature

Hydrocarbon degrading bacteria are known to be sensitive to pH in the soil, which may depend on biotic factors such as nutrients and water (Margesin and Schinner, 2001). The optimum pH value for oil degradation is 6.5 to 8.0 and the condition required for microbial activities to thrive is between 5.5 to 8.8 (Vidali, 2001). Thus, similar values were observed in all the soil samples during the bioremediation process in experiments (a) and (b), except in the initial stage of the treatment in experiment (b) where the addition of the diesel had a slight effect on soil pH. Soils affected by crude oil are expected to have a lower pH value than similar unaffected soils. The reason for low pH is that hydrocarbons may be expected to have free cations giving them the properties of a weak acid (U.S DOE, 1996). However, the addition of BSG and other factors such as temperature were seen to raise the pH level. This suggests that BSG enhances the compounds that neutralise the acidity of the soil brought about by the diesel contamination as reported by U.S DOE (1996).

Changes in temperatures are known to affect the utilization of substrate within hydrocarbon mixtures (Mittal and Singh, 2009) and also temperature changes the

rate of carbon mineralization, through the reduction of alternative electron acceptors (Van Hulzen, 1999). The temperature required for microbial activities range from 14 to 31^0C and the optimum value of temperature for oil degradation is 20^0C to 30^0C (Vidali, 2001). A similar temperature was recorded in both experiments with a slight variation at the beginning of the experiment as indicated in section 4.3.4 and 4.5.5. The temperature condition in the study could be regarded as warm, which is known to favour biologically driven degradation of compounds such as hydrocarbons (CL: AIRE, 2007).

More so, microbes that are known to degrade hydrocarbons needs oxygen to survive, in the study oxygen levels were not measured directly but a sufficient level of soil aeration was achieved by turning the soil and the moisture content was maintained accordingly. Thus, turning the soil enables the diesel and the soil/BSG mixture to mix and be evenly distributed, as expected this enhances the process by providing an enabling environment for the microbe colonies to have viable contact with food and nutrients. Hence in these experiments conditions were optimised which is something that may not happen in the field where environmental conditions may impact on the success of achieving a bioremediation target. It has been reported that bioremediation was delayed at a site in the U.S. by high moisture conditions due to unseasonably wet weather from November 1995, until June 1996 and soil temperature below 5^0C or above 32^0C could retard or stop microbial processes (U.S. DOE, 1996).

In the study here a consistent volume of water was added to the soil to maintain the experiment and in all cases this was 85 cms^3 for treatment without BSG and 176 cms^3 for treatment with BSG in 1000 g of soil, which is less than the field water holding capacity. The level of water added showed that the soil was moist but not waterlogged and because water was added twice weekly, in addition to turning the soil, the bacteria in the soil were expected to have a viable contact with the contaminants. However, the use of sufficient water to meet the water holding capacity and maintaining all environmental conditions may further enhance the breakdown of the diesel contaminants. Further studies are needed to demonstrate the practicality of the technique under field condition.

4.6.3 Total petroleum hydrocarbon (TPH)

Petroleum hydrocarbons such as diesel can be identified and quantified by comparing sample chromatograms with calibration of the gas chromatograph with diesel. Bento, (2005) calculated the percentage degradation of diesel oil as (TPH control – TPH treatment/TPH control x 100) and the initial hydrocarbon values establish the baseline or the starting point for a bioremediation project (U.S. DOE, 1996).

In the present study the progress of bioremediation was measured by calculating the percentage degradation of the diesel contaminated soil by measuring the total peak height and this was achieved by obtaining the initial hydrocarbon value, which served as the starting point and measuring it with the progress of the bioremediation process up to the last day of the experiment. From both experiments in Fig 4.1 and 4.2 there was an increasing reduction in the TPH using the total peak height in all treatments with BSG/consortium and treatments without BSG. There are disadvantages of using the total peak because it is difficult to determine the mass of carbon, however, it could be used to calculate the percentage degradation and compared to the extract from the chromatogram to show if degradation actually occurred.

In the study here percentage reduction of total peak height was used for the amount of diesel in the soil. Initially it has been evaluated that 68% of diesel can be recovered from the soil according to the method in section 3.7.2 and Fig.3.4. This is the amount of diesel that can be recovered in the soil when a given amount is added. The initial soil concentrations of the diesel were 5,000 mg kg^{-1} and 10,000 mg kg^{-1} for experiment (a) and (b) (Table 4.1 and 4.6), however, given the 68% recovery level the measurable concentration of diesel in the soil for experiments (a) and (b) were 3,400 mg kg^{-1} and 6,800 mg kg^{-1} respectively. To this end the percentage reduction and the degradation were based on these concentration levels for both experiments.

In experiment (a) TPH reduction was measured by the total peak height in all the treatment types from day 14, 28, 42 and 60. This was calculated as percentage degradation as shown in Figure 4.1. The percentage degradation from experiment (a) showed that at day 60 the treatment with soil plus diesel recorded 72%

degradation and the addition of consortium resulted in 83% degradation while the addition of BSG resulted to further degradation of 95%. In experiment (b) TPH reduction was also calculated as percentage degradation and reduction was observed in all treatments at day 28, 42, 60, 74, 90 and 104 as indicated in Figure 4.2. The qualitative observation of the chromatogram for treated soil plus diesel and treated soil plus diesel plus BSG are shown in Figure.4.3 and Figure 4.4 in the results section shows that the lower molecular weight molecules breakdown more quickly than those with a high molecular weight, which are considered to be recalcitrant. The treatment with soil plus diesel plus BSG resulted in 95% degradation. The statistical analysis for both treatments showed that there was a significant difference between the treated soil with BSG and treated soil without BSG $p = 0.05$.

The results from experiment (a) and (b) showed the effective bioremediation of petroleum contamination. This is contrary to the study of Bento *et a*l. (2005) which carried out a comparative evaluation of soil contaminated with diesel collected from Long Beach, California, USA and Hong Kong and China. The three technologies of natural attenuation, biostimulation and bioaugmentation were evaluated. Natural attenuation was the ability of the microbes present in the soil to degrade the contaminants. Whilst biostimulation involved the addition of $(NH_4)_2$ and K_2HPO_4 and bioaugmentation was the addition of a microbial consortium from selected species isolated from a contaminated soil.

After 12 weeks of incubation all three treatment types showed different effects on the degradation of TPH with bioaugmentation showing the greatest degradation in Long beach soil and Hong Kong soil. Natural attenuation was more effective than biostimuation (addition of nutrients). The light fraction ($C_{12} – C_{23}$) degradation using bioaugmentation in the Long Beach soil was 75% and biostimulation and natural attenuation were 46% and 49% respectively. Whilst in the Hong Kong study, soil bioaugmentation was 73% and biostimulation and natural attenuation were 45% and 46% accordingly.

In the present study biostimulation (addition of BSG) in experiment (a) was more effective than bioaugmentation (addition of consortium) which was a treatment without BSG. The reasons for this could be attributed to different conditions and

experimental design as the hydrocarbon degrading bacteria consortium used by Bento *et al.* (2005) were those previously isolated from Long Beach, California. In the present experiment the bacterial consortium used was obtained from Teesside University and was known to be hydrocarbon degrading bacteria and the soil was a spiked soil as compared to contaminated diesel oil obtained from the field.

In the results reported here the BSG may optimise the conditions by the addition of organic nutrients as suggested by Dadrasnia and Agamuthu, (2013). In the study reported by Dadrasnia and Agamuthu, (2013) tea leaf, soy cake, and potato skin were used to augment the bioremediation of soil spiked with diesel for a period of 3 months in the laboratory. Their findings showed that hydrocarbon utilizing bacteria were more abundant in the diesel polluted soil amended with different organic wastes than of the unamended polluted soil.

Another study conducted by Ameh *et a*l. (2012) investigated the effect of earthworms on the bioremediation of soil contaminated with used engine oil amended with poultry manure. The results of the study showed that the biodegradation rate of the oil concentration of 5, 10, 15 and 20 g/kg of soil corresponded to equivalent degradation of TPH by 16.91, 20.82, 34.68 and 36.28% respectively after 42 days of treatment. These results can be compared to the use of biodegradable waste to augment the bioremediation process. In this study experiment (a) and (b) showed improved breakdown of TPH with addition of BSG which was previously calculated to be 95% in 60 and 104 days of study in experiment (a) and (b) respectively.

However, oil bioremediation is limited by the availability of nitrogen and phosphorus (Yang *et al*. 2009) and it has been suggested that the ratio of C: N: P required for effective bioremediation was 120:10:1 (Ghazali *et al.* 2004) and in soils contaminated with diesel the ratio TPH:N is 150:1 indicating that these soils are deficient in nitrogen (Deuel, 1994). BSG which is known to have a high nutrient content (Thomas and Rahman, 2006) could be the source of nutrients for microorganisms.

In terms of hydrocarbon breakdown oil degraders are in most cases bacteria that adapt to use these hydrocarbons as a carbon and energy source. In the study reported here the micro-organisms may be capable of using the diesel as a food source however, unless growth conditions are optimal, bacterial adaptions can be

slow and the degradation results are poor (Kauppi *et al.* 2011). The capacity of a microbial population to degrade pollutants within an environmental matrix can be enhanced by the addition of specific microorganisms to enhance the local population or by the optimisation of the soil condition for the microbial populations present (Fantroussi and Agathos, 2005). Bioaugmentation (addition of consortium) adopted in the study involved two treatment types with one involving the mixing of the consortium with soil and diesel and the other mixing the consortium with soil, diesel and BSG. The result of the mixture of the consortium with BSG had the same percentage degradation of TPH (95%) as the biostimuation in experiment (a).

In the results reported here BSG may optimise the conditions by the addition of inorganic nutrients as suggested by Abioye (2012). This was seen in experiment (a) when BSG was added to the soil plus diesel plus consortium and experiment (b) where BSG was added to the soil plus diesel. Hydrocarbon degrading bacteria were observed to be significantly high, hence giving a greater reduction of TPH compared to the treatments without BSG.

4.7 Conclusions

The objective of bioremediation is to naturally remove the contaminants from the soil in an environmentally safe manner by harnessing the efficiencies of biological, physical and chemical processes in the soil. In order to establish the efficiencies of the bioremediation process, the results of chemical and microbial data were used in this study as a line of evidence of bioremediation degradation.

The functional responses of the soil microbial activities in the treatments with BSG showed the dynamics of the bacteria in the soil as indicated by the oil agar in both experiments. This suggests that the BSG is a potential source of nutrients in augmenting the bioremediation process in that it has appreciable quantities of nitrogen and phosphorus, which are necessary nutrients for biodegradation activities (Adesodun and Mbagwu, 2008). When significant amounts of organic residues are added to polluted soil such as hydrocarbon there would be an increase in oxygen diffusion, porosity of the soil and adequate pH (Semple *et al.* 2001).

The BSG is associated with high water and nitrogen contents, which is likely to supply nutrients to the microbial population present in the diesel contaminated soil,

thereby enabling the microorganisms to have a viable contact with the contaminants. The BSG may also bring additional bacteria that help in the degradation process and serve as a bulking agent to improve oxygen diffusion and may immobilize a greater quantity of bacteria cells. This may be the reason for the increased breakdown and degradation of TPH in treated soil with BSG.

In this study the application of the BSG and proper maintenance of the environmental conditions were essential aspects for treating the soil. The results of the microbial activities and chemical analysis could be used for prediction of bioremediation of diesel contaminated soil using BSG as a nutrient, which is likely to be the limiting factor in oil biodegradation (Nichols and Venosa, 2008).

The results presented in this chapter indicated that the use of BSG would reduce the timeframe for bioremediation hence making it a more attractive option. There was a greater growth of micro-organisms on oil agar indicating the presence of bacteria capable of breaking down diesel after treatment with BSG. The use of BSG to aid the bioremediation process may be due to it bringing populations of micro-organisms that readily adapt to the use of diesel as a foodstuff. In the study here it improved the reduction of diesel concentration of 3,400 mg kg^{-1} and 6,800 mg kg^{-1} by 95% in experiment (a) and (b) respectively.

The aim of the laboratory feasibility study is to identify limiting factors and recommend ways to mitigate these limitations in the field. In this study the laboratory experiment is potentially useful because it would determine how to enhance the rate of biodegradation of the diesel contaminated soil under controlled conditions. The results can be used to provide information on estimated cost and duration of the treatment.

The next chapters will evaluate the economic and environmental costs with and without BSG based upon the timeframe for TPH reduction that have been established in this chapter.

Chapter 5

IS THE USE OF BSG IN BIOREMEDIATION COST-EFFECTIVE?

5.1 Introduction

The aim of this chapter is determine the economic costs of using BSG to remediate soil contaminated with diesel and make comparisons to the costs of other remediation options to be considered in the study. The sections covered include the use of BSG, discussions with the remediation expert and developing the case study site. The chapter further describes methods used to derive the various costs used in the study including justification for the costs adopted, sensitivity analysis, results, discussions and conclusions.

5.1.1 The use of BSG to remediate diesel contaminated soil

The use of BSG as a nutrient to augment the bioremediation of contaminated land has been shown to be successful in the laboratory (Chapter 3) and as such is a promising technique both for the remediation of hydrocarbon contaminated soils and as an alternative disposal option for the BSG. The technique is relatively simple and the BSG may bring additional nutrients to the bioremediation process. However, it would only be used in practice if the technique was found to be economically feasible.

As the continued development of greenfield land is now considered to be unsustainable and the reuse of brownfield sites has provided an alternative for residential or commercial developments (Atkinson and Doick, 2010), the use of bioremediation could provide a viable option for the regeneration and redevelopment of contaminated land and the construction of low cost urban housing units. The use of BSG to augment the process could reduce the time taken for the biological breakdown to occur. Stakeholders in the U.K. remediation industry are becoming increasingly focused on the economic costs of remediating contaminated land and this is seen as one of the major drivers in remediating contaminated land (SuRF-, 2010).

There are some 300,000 ha of brownfield land, identified as posing a risk to both human health and the wider environment due to contamination, including

hydrocarbon pollution, in the U.K. (Dixon *et al.* 2007). In addition the U.K., Government set a target of 151,031 new homes to be built from 2013 to 2033 in the U.K., based on 2008-based population projections (CLG, 2012) and most of these homes are to be built on brownfield sites. Hence there may be a need to build upon contaminated land in the future and for the contamination to be remediated using a method that is economically feasible.

Due to the importance of economic cost as one of the major drivers of remediation of contaminated land (U.S. EPA, 1997), this study will carry out an assessment of the total costs of using BSG to remediate contaminated land. This cost will then be compared to the economic costs of other remediation options.

The following section will determine the cost of bioremediation using BSG and compare the costs to other remediation methods. Firstly, a conceptual site model will be devised representing a typical site in the U.K. The model will then be used to determine the financial costs associated with bioremediation, with and without BSG, together with a range of commonly used remediation options. The case study was devised after consultation with a range of practitioners in the field of contaminated land.

5.1.2 Discussions with the remediation expert and case study site development

The economic costs utilised in the study were obtained from remediation practitioner Grant Richardson referred to in this study as Pers.comm. Grant is a remediation expert with 20 years of experience in a multi-disciplinary environmental consultancy in the North East of England, working on contaminated land projects throughout the U.K. He has undertaken remediation works in the areas of brownfield sites, the petrochemical industry, petrol stations, landfill, scrap yards and former gas works, including on-site treatment of fuel, solvent and chemical spillages.

A discussion with the remediation practitioner (Pers.comm, 2011), showed there was a need to devise a hypothetical case study site, typical of a contaminated land site in the U.K., that would be representative of all costs associated with the different remediation methods to be considered in the study. A literature search found that there are limited studies which address the issue of remediation costs therefore this

study seeks to determine the true financial costs associated with bioremediation, with and without BSG. In addition remediation costs are strongly site-specific with variability of geological, hydrological and chemical factors having large impacts (SuRF-2010). Costs are also known to be influenced by how stringent the remediation targets are and the differences in remediation targets could be affected by the duration and impacts that may arise from the remediation methods (Pers.comm, 2011).

In the discussions it was suggested that the case study site should be approximately 10 ha in size and the volume of contaminated soil should be 20,000 tonnes with a total mass of 20,000 kg of diesel. This type of site is typical of many sites across the U.K. and that was the reason for its adoption in this study. In order to clean-up the site six different remediation methods were considered: bioremediation with and without BSG, natural attenuation, landfill, SVE and thermal desorption. Sensitivity analysis was carried out to determine the variation in input data. This will allow the indicators used in carrying out the sensitivity analysis to hold a wider confidence margin and enable the differences in measuring the impacts of various remediation methods to be considered.

In order to account for the direct and indirect costs the proposed end use of the site would be the development of 300 new homes, the site will be based in Sunderland. The economic costs including hidden costs such as the time delay for development, which is not normally considered during site investigation will be evaluated and costed. Other remediation costs were derived from past and present company reports, journals and current remediation works.

The conclusions drawn from the case study site showed that remediation costs are site specific as costs at one site may not be used for other sites, however, some costs relevant to one site may be useful in costing other remediation projects. The cost model that has been developed could be used for similar sites provided the conditions and the assumptions are the same. It cannot be generalised for all sites. More so, the inclusion of indirect costs allows the time delay in any remediation project to be evaluated and could be used to calculate the hidden costs associated with different remediation techniques.

5.1.3 Developing the case study site

In developing the case study, to be evaluated in this project, the site was assumed to be approximately 10 ha in size with significant levels of hydrocarbon contamination that was identified underlying the area of the site. The approximate volume of contaminated soil is 20,000 tonnes with a total mass of up to 20,000 kg of diesel. It is an industrial site and the contaminated layer is to a depth of 10m. The site is known to have had a range of uses including: fuel depot, transport yard, & warehousing, it is now derelict and abandoned. An underground pipe which was corroded has left a significant volume of hydrocarbon contamination in the soil. This type of site is typical of many sites across the U.K. (Pers.comm, 2011, CL:AIRE, 2011). A developer has recently purchased the site and intends to build 300 new houses

The project is set to regenerate the area with new housing being built. The area already has housing surrounding it. The cost of acquiring the 10 ha of land similar to the one described in the case study site, without contamination, in the U.K. is about £1.5m depending on the location (Pers.comm, 2011 and U.K Land Directory, 2011). However, with higher levels of contamination the value of the land would have been reduced or acquired at no cost. The site preliminary conceptual model (Figure 5.1) highlights the potential sources of contamination, pathways and receptors. The requirement for the remediation of the site is limited to work necessary to prevent an unacceptable risk to human health and the environment. The potential receptors of the contaminants are residents, construction workers onsite, groundwater and the nearby river. The site conceptual model shows the damaged underground tanks from the former petrol station, which is close to residential properties and surface water with potential receptors of the diesel contaminant. Although the diesel contaminants do not affect groundwater, there is possibility of the vapour leaking down through the saturated zone, which may lead to an impact on the natural ecosystem.

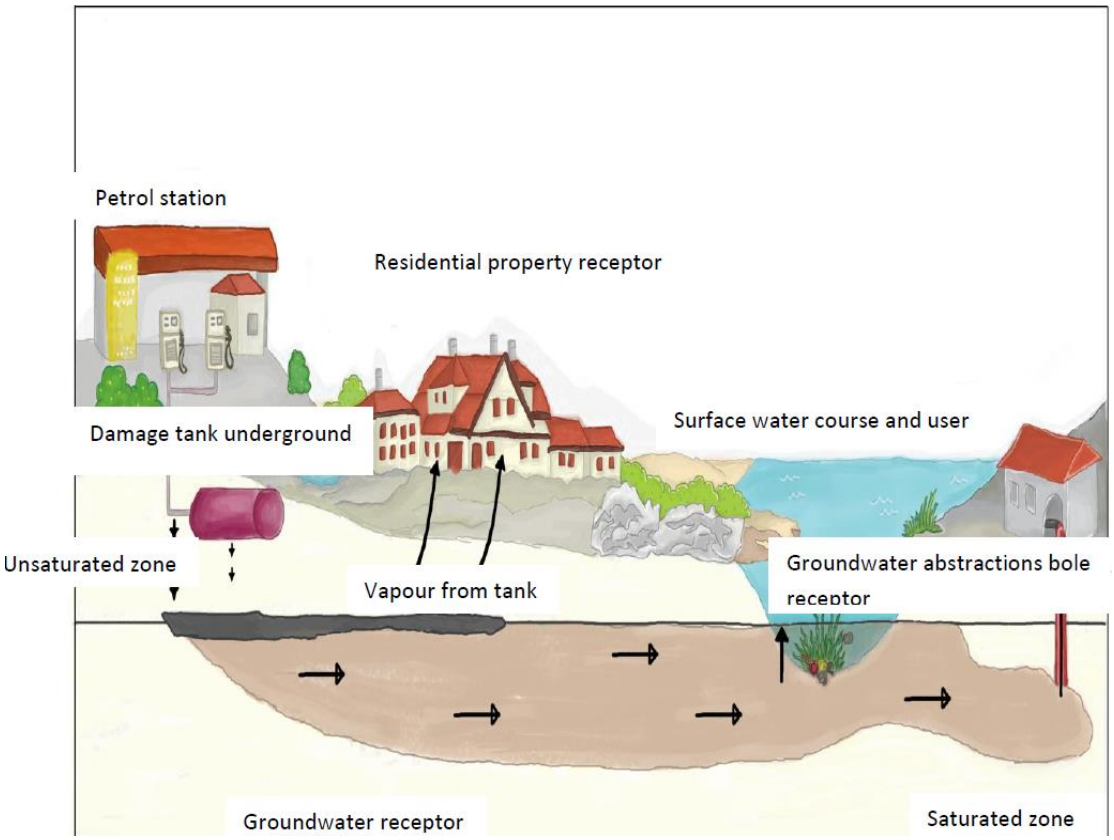

Figure 5.1: Conceptual site model for the hypothetical site to be used in the research.

In remediating the land for residential purposes, the economic cost for five remediation options will be evaluated. These include bioremediation (with BSG), bioremediation (without BSG), landfill, thermal desorption, natural attenuation and SVE. The scenario (Figure 5.1) is a typical conceptual contaminated land model in the U.K. with potential pathways for the pollution being evaporation and the movement of vapours into the housing, movement of any soluble pollutants into the groundwater and into the river together with contamination of drinking water abstracted from the river and the groundwater. Identification of these pathways means that the site would need to be remediated.

The conceptual site model serves as the foundation for evaluating the restoration potential of the site and the effectiveness of operating the remediation systems considered. The goal of site remediation is to find the most cost-effective method of

reducing present and future risk by combining the three risk reduction techniques:

- Source reduction – Achieved by employing the six remediation options such as bioremediation with and without BSG, natural attenuation, SVE, thermal desorption and landfill disposal option.
- Pathway elimination – Examples include vapour collection systems with the appropriate technology (off-gas facility), collection of leachate, pumping to stop the migration of contaminants towards down- gradient receptors such as land and water.
- Protect receptors – Land use controls such as site fencing, surface capping, digging restrictions, protective clothing, and groundwater well restoration.

Although the complexity of risk assessment will vary from one remediation method to another, there are several common elements that should be included in every conceptual site model. These are similar to the three listed above, Table 5.1 shows the six remediation options considered in the study and the details of their remediation strategy. A risk assessment model is developed for the site in section 5.1.3 and appendix IV.

Techniques	Details
Bioremediation (with BSG)	The use of brewery spent grain to augment the bioremediation processes.
Bioremediation (without BSG)	The modification of the environmental condition of the soil by adding water and turning the soil to ensure the microbes act on the contaminants
Landfill	The contaminated soil will be excavated followed by disposal to a suitable landfill, the excavated soil will be replaced with a certified subsoil and topsoil.
Natural attenuation	No attempt to remove contaminants or to contain the contaminant prior to redeveloping the site. Soil will be left at the site until concentrations have reached an acceptable level.
Soil vapour extraction (SVE)	Soil vapour extraction is an in-situ technique which induces an airflow through soil to enhance the volatilisation of the organic contaminants and aerobic biodegradation of the contaminants.
In-situ Thermal desorption	Thermal desorption is principally a thermally induced physical separation process where contaminants are vaporised from a solid matrix and transferred into a gas stream where they are easily treated or managed.

Table 5.1: Five potential remediation options adopted for the case study site.

Table 5.1 gives details of the remediation options that will be evaluated in this study. Bioremediation with and without BSG will be compared to disposal to landfill ‘dig and dump’ the option most often used for a small sites of this nature (Pers.comm, 2011), SVE, thermal desorption and the do nothing option, natural attenuation.

5.2. Methods

Any remediation project begins with a feasibility study, which determines the necessary design for an effective remediation strategy. It has been assumed that the initial site investigation has recommended the six options in Table 5.1. The cost of the initial site investigation for this study was obtained from a remediation practitioner who recommended that for a 10 ha site the costs would have been £1-2m (Pers.comm, 2011). This cost will be the same for the six remediation techniques being evaluated in this chapter.

The various values utilised to evaluate the different remediation options are presented in Table 5.2. Most values were derived from the remediation practitioner (Pers.comm, 2011), which include the cost of disposing of the soil and re-filling the land, cost of labour during remediation work, cost of sampling the soil and the number of samples for the site. Some values in Table 5.2 were also obtained from a company report (Sirius, 2010) these included: the time frame for natural attenuation to be completed and the type of machinery used including the diesel consumption per day during remediation work.

No	Data	Sources and date
1	10 ha land cost of £1-2M for site investigation with equivalent 20,000 tonnes of soil (10,000 m^3)	Pers.comm, 2011- meeting (26-08-2011)
2	Number of new houses that could be developed on a 10 ha Brownfield site – 300 Units	Waste &Resources Action program (WRAP, 2008); Pers.comm, 2011- meeting
3	£540.00/ month rent for 3 bed unit from 0.1 to 1.5 miles from Sunderland City Centre.	http://www.rightmove.co.uk/property-to-rent/Sunderland.html (22-07-2011)
4	Cost of supervision for Foreman or Surveyor @£900.00/ week	Pers.comm, 2011 –meeting (02-07-2011)
5	**Cost of obtaining spent grain** Cost price of £38.00/tonne from supplier And £4.50/tonne for transporting BSG in the UK taking into consideration fuel cost, including driver and distance covered (haulage costs)	http://www.dairyco.org.uk/datum/farm-inputs/feed-prices/uk-feed-prices.aspx Ben-Hamed et al.(2012)
6	£100.00 every 100m^3 for the cost of chemical analysis in addition of £1.00 for the site plus cost of interpreting and reporting (personnel cost).	Pers.comm, 2011 –meeting (26-08-2011)
7	14tonne excavator per day will use 70 litres diesel at the current average cost of £1.40/ litre	Sirius Report 2011 (03-02-2010)
8	Welfare cost of £500.00 per month for the 10 ha site.	Pers.comm, 2011- meeting
9	**Cost of disposal to landfill** Soil handling cost £1.00/tonne, haulage cost £6.00/tonne, disposal cost £30.00/tonne and landfill tax cost £48.00/tonne and additional 20% overhead costs. The total cost is £102.00/tonne. Distance from Sunderland to Hartlepool @30 miles (48.28 km)	Sanders and Vernon, 2009 ; Pers.comm, 2011- meeting
10	**Cost of re-filling the soil** Topsoil cost £12.50/tonne, tilling and turning soil including labour £2.00/tonne, transporting the soil cost £5.00/tonne. The total cost of re-filling the soil £19.50.00/tonne	Pers.comm, 2011- meeting http://archive.defra.gov.uk/environment/waste/strategy/factsheets/la
11	**Natural attenuation duration and sampling** Time for natural attenuation between 3-8 years and should not take more than one generation (15-30 years) Natural attenuation sampling once in every month and expected to last for 5 years.	Pers.comm, 2011- meeting; Sirius report, 2010 and Defra report, 2005

12	Sampling Brownfield site every 2 weeks during remediation work	WRAP, 2009: Project code OBF001-042;
13	Number of sampling of Brownfield soils for TPH should be 1 @100 m^3	YAHPAC, 2010 version 1.2 and Pers.comm, 2011- meeting
14	30 miles from Sunderland to Cleveland in Hartlepool nearest landfill site	Pers.comm, 2011- meeting; www.augeanplc.com
15	**Cost of remediation for SVE (In-Situ)** When >5000 m^3 of soil remediated @£100.00 When < 5000 m^3 of soil remediated @ £400.00 SVE in situ projects are typically completed in 1-3 years	Defra, report (2010), : Davis, L., 2009 and FRTR (URL4.1)
16	Field trial of the time scale for soil vapour extraction in UK was 325 days (11 months)	CL: AIRE TDP 28, 2011
17	SVE treatment typically completed in 3 years	Baker et al., 2008
	Cost of remediation using Thermal desorption (In-Situ) When >5000 m^3 of soil remediated @£144.00 When < 5000 m^3 of soil remediated @ £168.00	Defra, report (2010) : Davis, L., 2009.
18	Thermal desorption projects are implemented less than 12 months (In situ)	Defra, report (2010), FRTR, 2007 and Nathanial et al., 2007
19	Using thermal desorption to remediate 11,100 m^3 of soil using 147 heater wells for 169 days (5.6 months)	Baker et al., 2008

Table 5.2: Data and sources of information used to derive the economic costs presented in this chapter.

The site was assumed to be approximately 10ha with about 10,000 m^3 of soil that needed to be remediated. The purpose of the clean-up was for the site to be redeveloped for low cost housing units. The average cost of 3 bed units located between 0.1 to 1.5 miles from Sunderland city centre was estimated to be £540.00/ month.

Also included in the cost analysis was the cost of obtaining BSG taking into consideration cost, labour, haulage and distance from Hartlepool to Sunderland. Other costs considered were cost of supervision for foreman or surveyor, cost of soil handling or haulage to dispose or refill the land, current landfill tax rate and the overhead costs when the landfill disposal option was considered.

The cost of SVE and thermal desorption were determined from a recent Defra report on the average cost of implementing SVE and thermal desorption in the U.K. These

costs also take into consideration the minimum and maximum costs for soil quantities above and below 5,000 m^3. The time frame for thermal and SVE methods were derived from the same Defra report together with other completed remediation projects (Baker *et al*. 2008). The next section provides detailed justification of the values and costs used in the study reported here.

5.2.1 Justification for the values and costs adopted

The main aim of this research is to investigate the use of BSG to augment the bioremediation process 'Enhanced bioremediation'. Bioremediation is currently considered as a cost-effective technique (Sayara *et al.* 2011) as it does not need engineering technology to remove the contaminants.

The following section provides the justifications for the values used in the assessment of the economic costs presented in this chapter (Table 5.2).

a. Costs associated with delayed development of the land

Bioremediation is a widely used technology and is often considered to be simple and cost-effective when compared to other remediation methods (American Petroleum Institute, 1983). It has been successfully applied in both the laboratory and field studies and is particularly effective at degrading petroleum contamination (Balba *et al.* 1998). However, bioremediation takes time and there will be costs associated with the delayed development of the land.

In this chapter the time taken for hydrocarbon reduction to reach levels acceptable to the regulators has been estimated as six months. This value is based upon the results of the laboratory studies, reported in Chapter 3, together with corroboration from other studies on the reduction of hydrocarbon contaminated soil to a level allowing the re-use of the soil. However, this is a general estimate as bioremediation is a site and season specific process and pilot studies are a pre-requisite for a full scale remediation application (Balba *et al.* 1998). It has also been reported that field-scale trials of bioremediation, for a hydrocarbon contaminated site in the U.K., take three times as long, to achieve the same endpoint, as trials using the same soil carried out under laboratory conditions (Diplock *et al.* 2009).

In the laboratory studies conducted for this study there was a reduction of 92-95 % of

TPH in 60 to 90 days when BSG was added and 72 to 90% when no BSG was applied (Chapter 3). Therefore, using the factor of three reported by Diplock *et al.* (2009) the time frame taken to reduce the hydrocarbon content of the soil to an acceptable level would be 6 months (with BSG) and 9 months (in the absence of BSG).

The cost of not developing the land during the period of remediation could be calculated based upon the rentable value that would have been accrued, over the period, if the housing units had been built. Currently the average rental value for 3 bed room units in Sunderland is £540.00/month (Table 5.2) for housing units situated between 0.5 and 1.5 miles from the city centre where the case study site is situated.

As with bioremediation natural attenuation is also site specific as it depends on the contaminants involved, the level of contaminants, environmental condition, the amount of soil and other factors (EA, 2004). As the present study is based on a hydrocarbon contaminated site a 5 years baseline will be adopted as the time frame to achieve remediation objectives for natural attenuation. The 5 years baseline was chosen because Sirius (Table 5.2) has an on-going remediation project associated with a hydrocarbon contaminated site; it was initially estimated to be completed in 3 years but later projected to be completed in 5 years' time based on the line of evidence of the degradation. Although monitoring the natural attenuation of contaminated land is expected to be achieved within a reasonable timescale. The remediation objective should be achieved in less than one generation or 30 years (EA, 2004).

The cost of the delayed development associated with natural attenuation can then be calculated assuming a rental value for the housing of £540/month (Table 5.2).The next remediation strategy considered in this chapter is excavation of the soil and its disposal in a designated landfill. In terms of delayed development there would be financial costs associated with this option as it is assumed that there would be time delays associated with disposing and re-filling the earth with the amount of soil that would be removed in this case 20,000 tonnes (Table 5.2).

The cost of the delayed development associated with landfill disposal option can then be calculated assuming a rental value for the housing of £540/month (Table 5.2). The soil will be excavated and disposed in a designated landfill as not all

landfills will be willing to accept hazardous waste. The closest landfill to Sunderland is in Hartlepool (Pers.comm, 2011) and it is 30 mile drive. Disposing of the soil to landfill will require a 16 tonne lorry, which will require 6 trips/day, equivalent to 96/tonnes/day so to dispose of 20,000 tonnes of the hazardous waste will take 208 days.

Therefore, if more lorries are used for example 4 lorries, 384 tonnes of soil will be disposed of daily. The time taken would be 8 weeks. The earth would be replaced with the same amount of soil, which will take a similar number of days with total time of 16 weeks (4 months). The renting value for this period of time will be apportioned to the delay in disposing and replacing the soil, given previous calculation of £540.00 for 3 bed rooms in Sunderland (Table 5.2).

The duration of operation and maintenance of SVE for in-situ treatment is typically medium to long term and cleaning the site using SVE can take years (typically completed SVE projects take 1 to 3 years) (EPA, 2001, FRTR, 2012 and Defra, table 5.2). However, in a recent trial undertaken by Contaminated Land: Application in Real Environment (CL: AIRE) 2011 in Kent in the United Kingdom to assess the effectiveness and the commercial viability of combining SVE with in situ frequency heating technology (ISRFH), in order to remove volatile (hydrocarbons) and semi-volatile organics from the soil, it was found that a combination of SVE + ISRFH, which was operated intermittently, led to the achievement of the removal of the hydrocarbons from the soil in 60 days at a cost of £194/m^3 and SVE alone removed the hydrocarbon in 325 days at a cost of £174/m^3.

It has also been reported that the type of soil and the volume of harmful chemicals present will determine how long it will take to implement an in situ SVE system (U.S. EPA, 2001). Typical SVE technologies achieve clean-up within 3 years (U.S. EPA, 2001, U.S.EPA, 2008a and Defra, 2010) with the time taken being dependent on the remediation activities such as work plan preparation, equipment mobilization, subsurface installation, above surface installation, start-up/operation and demobilization and site restoration.

One of the limitations of SVE is the off-gas treatment needed: this is due to the potential high cost of energy to heat the incoming SVE gas. In a series of studies conducted by Bostrom, (2004) in the USA on the cost of energy for a catalytic

oxidizer, typical annual energy cost for the catalytic oxidizer system, operating at 100 to 200 standard cubic feet per minutes (scfm) ranges from $20 to $40/scfm for natural gas and from $50 to $100 per scfm for electricity heated system.

In another study in the USA, the operating cost of implementing a catalytic oxidizer with an off-gas flow rate of 250 scfm at maximum contaminant rate was $22.00/day. A total mass of 10,000 pounds of contaminants was destroyed in 5 years of remediation work and the daily cost of electricity was between $25 to $35/day. In a similar study the extracted flow from the subsurface was 230 scfm, with maximum contaminant extraction rate of 130 pounds/day for several weeks. During one year of operation, the oxidizer destroyed 16,000 pounds of gasoline with an average cost of $130/day. The project ran for over a year and the fuel cost for the period was $48,000 (U.S. EPA, 2006).

The SVE technology that would be adopted in this study will require heating of the air in order to enhance the volatilization of the diesel oil in the soil. Most petroleum products are biodegradable to a certain degree but heavier products may be difficult to remove. Some diesel compounds are less volatile and these compounds can be removed by injecting hot air to enhance their volatility (U.S. EPA, 2012a). This may result in additional costs for the SVE technique due to the high energy demand that may be required, especially in the U.K., where energy costs are high compared to the U.S. where the technique is more popular (William and Brankley, 2006). This will be a limiting factor for SVE and the extended time delay that could be associated with the technique may even prohibit the use of the SVE in cleaning-up soils. SVE may not be viable for diesel contaminated soil because some components of diesel are less volatile and may not readily be removed. That is why SVE technologies are most often used in conjunction with other remediation method such as bioremediation or natural attenuation in diesel contaminated sites - the volatile compounds are removed by SVE and the less volatile compounds are removed by bioremediation or natural attenuation.

However, in this study, the implementation of in situ SVE could be achieved in 3 years given the volume of soil that is involved and this is the Defra estimate in the U.K. for large volumes of soil (Defra, 2010). Therefore, the cost of not developing the land for 3 years will be calculated using the rental value that would have been

accrued if the housing units would have been built.

Remediating soil using thermal desorption may take from a few months to several years, as the time it takes are functions of the size and depth of the polluted area, the type of soil and the conditions present (Eugris, 2012). Thus, contaminant concentration has a huge impact on the time scale to achieve thermal remediation objectives. In a study conducted on a site used by the Navy and the National Aeronautics and Space Administration (NASA) in the U.S. with contaminants of approximately 55,607 lbs. (25,275.91kg), it took 279 days (9.3 months) to remove the volatile organic compounds and semi-volatile organic compound from the soil using thermal desorption (NAVFAC, 2007).

In another study a brownfield site of about 26 acres (10.52 ha), with contaminants estimated to be 86,000 lbs. (39,008.94 kg) in New York, USA was remediated and redeveloped using in situ thermal desorption (Heron *et al.* 2012). The dilapidated site was contaminated with volatile organic compounds and semi-volatile organic compounds and the total area of the site met the agreed clean-up standard within 320 days (11 months) of thermal treatment (Heron *et al.* 2012). A recent design to implement full scale thermal enhanced SVE for the remediation of the unsaturated zone of the soil in a former chloro-benzene process site in Illinois USA (Krummrich, 2011) with contaminants of 440,000 lbs. (199,580.64 kg) is anticipated to take 4 years from 2012 - July 2016.

Therefore, using thermal desorption technology could depend on the concentration of contaminants and other factors such as design technique, geotechnical properties of the soil, soil moisture content, and spacing between the heaters (Johnson *et al.* 2009). It has been estimated that the time scale to achieve the remediation objective for thermal desorption with all regulatory requirements fulfilled is less than 6 months for ex-situ thermal desorption and less than a year for in situ thermal desorption (NAVFAC, 1998, Nathanial *et al.* 2007). A typical ex-situ thermal desorption project in the U.S. takes about 4 months to be completed (U.S. EPA, 2008a).

In a study conducted in Denmark at Reerslev site, the selected technology was thermal remediation by in situ thermal desorption, with simultaneous application of conducting heating and SVE in removing dense non-aqueous phase liquids (Baker *et al*. 2011). In the study a soil volume of 11,100 m^3 was treated with 147 heater

wells and it took 169 days to achieve the remediation objective. The actual energy consumption during the life of the in situ thermal desorption was 342 kWh/m^3 and was close to the initial estimate made using life cycle assessment. The initial cost estimate of the life cycle assessment was 3.3 million Euros when 12,560 m^3 of soil was projected for remediation but the actual cost was 2.9 million Euros and 11,100 m^3 volume of soil was remediated.

In the study reported here a similar thermal desorption technology would be implemented to heat the 20,000 kg of contaminant concentration spreading over 10,000 m^3 in the treatment area. This level of contaminants could be treated for 8 months using a similar level of treatment to the schemes described at the Reerslev site. The 8 months estimated in the study would include 6 months for heating the soil and 2 months for demobilizing the equipment, screening the soil and treating the residuals of the captured gases (Johnson et al., 2009). More so, the 8 months duration falls within the 1 year Defra and Atkins company report expected time frame for in-situ thermal desorption projects to be completed in the U.K. (Defra, 2010 and Atkins report, 2007). Then the time it takes for the remediation activities to be completed will be evaluated in terms of rental value that would have been generated if the land was to be developed for the housing units. The rental value for a housing unit has previously estimated to be £540.00/month.

b. Costs Associated with Disposal to Landfill

The costs associated with the disposal of the contaminated soil and replacement with clean soil at the site can also be determined. The closest landfill, accepting hazardous waste, is in Cleveland Hartlepool, owned by Augean plc at Port Clarence (Table 5.2) a distance of 30 miles from Sunderland. For the volume of contaminated soil at the site in Sunderland a total of 16 vehicles will require 1,250 trips to dispose of the 20,000 tonnes of soil from the site.

Costs associated with landfill disposal include the cost of excavation, transporting the soil and landfill costs. The current costs obtained from a remediation practitioner (Pers.comm, 2011) include £1/tonne for the cost of tipping/handling the soil, £6/tonne for haulage, £30/tonne for disposal and the landfill tax for hazardous waste which stands at £48.00/tonne in 2011 plus an additional 20% overhead costs (Table 5.2). The total cost derived from this estimate is £102.00/tonne to dispose of the soil to

landfill.

In addition topsoil would be needed to fill the areas of the site where the contaminated soil has been removed. The topsoil is assumed to be obtained from a single source, which has been inspected, tested and approved in advance by the supervising engineer.

As 20,000 tonnes of contaminated soil was removed from the site the site would need to be re-filled with the equivalent amount of soil. The topsoil that would be required will be 20,000 tonnes and it will cost £12.50/tonne in addition to £5/tonne to transport the soil to site assuming all soils would be obtained from the same location.

The earthwork and infilling of the soil will be conducted under the supervision of a foreman or supervising engineer at the cost of £900.00 for one week (Table 5.1). The earthwork machinery that would be used for infilling the soil together with labour costs is at a rate of £2.00/tonne and earth working is expected to last for 8 weeks (Pers.comm, 2011). Therefore the total cost of topsoil at the site will be £19.50/tonne (£12.50+£5.00+£2.00).

In similar circumstances if the same 16 tonnes lorry loads are used to dispose of the soil, the earth would be replaced with the same amount of soil, as such will take similar number of days to fill the earth, which is equivalent to 8 weeks (2 months). The total number of weeks for both disposal and re-filling is 16 weeks (4 months). The rentable value for this period of time will be apportioned to the delay in disposing and replacing the soil, given the previous calculation of £540.00 for 3 bed rooms in Sunderland (Table 5.1). The total cost would be £648,000.00 for the 300 housing units.

c. Costs associated with the use of BSG

The total costs of obtaining the BSG include the cost of buying the BSG and the cost of transporting it to the site for use in bioremediation. Brewery firms are located all over the U.K. and the U.K. produces over 0.5 million tonnes of BSG every year as indicated in Chapter 2. The current average price of BSG at the time of compiling this report is £38.00/tonne excluding transport costs (Table 5.2).

Since BSG would be purchased for the remediation work the cost of transporting the

waste to the remediation site will be adopted from the model developed by Ben-Hamed, (2012). The model estimates £4.50/tonne/mile (Table 5.2), which takes into account the transport, labour costs, distance covered, type of vehicle used, and the fuel cost for transporting BSG within the U.K.

The brewery firm from which the BSG would be obtained is in Hartlepool, Camerons Brewery, this is a large brewery that produces almost 7,500 tonnes/week. The cost of £4.50/tonne is considered a reliable input into the project analysis, as the calculation recognises the current inflation value and all variable costs involved in transporting the BSG from the factory to the contaminated site (Ben-Hamed, 2012). The total volume of BSG required for the study was 2000 tonnes given 10% of 20,000 tonnes of contaminated soil that needs to be remediated (see Chapter 3).

d. Costs associated with monitoring the remediation process

Another cost that needs to be considered in the bioremediation process is the cost of sampling the soil during the bioremediation process. This includes sampling the soil for chemical and biological analysis. The regulatory standard (BS 10175, 2001) for the investigation of potentially contaminated sites recommends 1-4 samples per ha during the exploratory stages including desk studies and site walkover. However, when assessing the suitability of a site during the remediation process the samples should be carried out in a manner suited to the material being sampled and the contaminants involved and there is need to agree with the local authority on how the sampling would be designed (YHPAC, 2010).The frequency of samples will depend on certain variables such as site history, heterogeneity of the contaminants, nutrient availability and the use of the land (BS 10175, 2001).

Therefore sampling for the study during the bioremediation process will be 1 sample per 100 m^3 (Table 5.2). This is according to the recommendation contained in the technical guidance for developers, landowners and consultants in the U.K. dealing with brownfield sites for TPH (YHPAC, 2010). Since there is 20,000 tonnes which is equivalent to 10,000 m^3 the site will require 100 samples to be undertaken every two weeks in order to monitor the degradation process. The two weeks' time frame for sampling the soil during the bioremediation process is in line with bioremediation of hydrocarbon contaminated brownfield sites in the U.K. (Pers.comm, 2011 and WRAP, 2009). Soil sampling would be carried out every two weeks for 16 weeks until

the remediation objective was achieved.

In a similar manner, a laboratory experiment was conducted in this study to monitor the degradation of hydrocarbons. Samples of the soil, for chemical analysis, were taken every two weeks throughout the bioremediation process until the remediation objective was achieved (see chapter 3). Therefore, the cost of £101.00 for every 100 m^3 of soil analysed (Table 5.2 Pers.comm, 2011 and EA, 2005) (including analysing, interpreting and reporting) will be applied to the 100 samples required every two weeks.

In order to demonstrate that natural attenuation is taking place the soil will need to be sampled and analysed at regular intervals. Natural attenuation undertaken on the on-going project by Sirius Limited (Sirius, 2010 Table 5.2) was carried out monthly and expected to continue for 5 years which is the time frame for the remediation objectives to be achieved. Sampling in the present study will be undertaken on a monthly basis for 3 years and when there is evidence of degradation it will be reduced to quarterly samples for the remaining two years. As such 100 samples will be collected from the site based on a sampling regime of 1 sample for every 100 m^3 at the costs of £101.00/100 m^3 to cover 10,000 m^3 including analysing, interpreting and reporting (Table 5.2).

e. Costs associated with energy use

The concentration of volatile organic compound extracted using SVE system would need continuous monitoring throughout the remediation period. Although the cost of in situ SVE is site specific and is a function of the size of the site, the nature and amount of the contaminants, and the hydrogeological setting (U.S. EPA, 1989). These factors invariably influence the number of wells to be installed, the blower capacity, the vacuum level required, and the length of time needed to clean up the site.

The cost of implementing SVE in the U.K. has previously been stated to be £174/m^3 when 480 m^3 of soil was cleaned up. But the cost range estimated by Defra (2010), for SVE in the U.K. is £400.00 when the volume of the soil is less than 5000 m^3 to be remediated and £100/m^3 when the volume of the soil is greater than 5000 m^3, as the volume of soil to be remediated increases the cost/m^3 decreases. This is one of the

advantages of SVE techniques (Sanders and Vernon, 2009). Therefore, the study will adopt the Defra cost for higher volumes as the quantity of soil that would be remediated in the study is 10,000 m^3.

Thermal treatments offer quick clean-up times with high energy demand (U.S.EPA, 2008a) and are typically the most costly treatment group. The heating of the soil requires a significant energy input and they are capital and operation & maintenance intensive. In the Reersler site in Denmark where thermal desorption was successfully used, the cost of the project was 2.9 million Euros including the cost of the energy. The volume of soil remediated was 11,100 m^3 and the heating period was 169 days. The initial life cycle analysis projected the volume of the soil to be 12,560 m^3 with an associated cost of 3.3 million Euros and the project was expected to be completed between 0.7 to 1 year. The remediation goal was achieved with 99.99 per cent of the contaminants removed and no health or safety issues recorded and all stakeholders including residents in the county were satisfied with the results of the remediation.

The Defra cost estimate was used in this study because the survey comprises of U.K. remediation practitioners, technical vendors and environmental consultants. The information provided is felt to be representative of the industry as a whole based on the size and experience of the companies that participated (Defra, 2010). In addition an estimate of thermal desorption, by remediation practitioners in the U.K., such as Atkins Company, have put the cost to be between £100-£250 for low heating system (Atkins report, 2007) similar to the type used in this study. As such the value of £144.00/m^3 by Defra would be better estimates of the cost of thermal desorption to be used in the present study.

f. Costs associated with management onsite

The labour and material costs of carrying out the bioremediation work include the cost of hiring a tractor, the costs of its operation, including labour and fuel, together with any welfare costs associated with running the facilities, throughout the period of the remediation work.

These costs will vary depending on the size and nature of the contractor carrying out the bioremediation. For instance, the remediation company might own the machinery needed on-site, or it might hire the necessary equipment for the time period of the

bioremediation operation. The purchase costs of the equipment used on site is high and some companies will hire it as and when needed. One other alternative used is to outsource the earth moving work to a sub-contractor.

If the total earth works are put together it will cost £1.00/tonne to turn the soil during the bioremediation process which includes the cost of labour, machinery, fuel and others (Sanders and Vernon, 2009). The size of the earth work equipment is approximately 14 tonnes consuming 70 litres of diesel/day (Sirius, 2010). This cost of £1.00/tonne will be adopted in this study because it takes all activities that entail the turning of the soil into consideration. Since there are 20,000 tonnes of soil that needs remediation the cost will be applied to this volume of soil.

Other costs that are associated with the bioremediation work are the welfare costs of running the facilities on site (organisation and maintenance costs). These include the cost of providing a telephone on site, canteen, toilet, costs of running a generator if there is no electricity and other administrative expenses. In a 10 ha contaminated site these costs were estimated to be £500.00/month (Table 5.2).

More so, there would be welfare costs associated with the disposal and re-filling of the site during landfill and this cost will be pro-rated according to the number of days the landfill process would take, in this case is 16 weeks (4 months) and the cost will be based on £500.00/month.

In conclusion the majority of costs used in this study were obtained from a remediation practitioner, industry experts through interview, conference presentations and workshop training and are the current prices in the remediation industry given the present economic conditions. Using costs from a practitioner provided an ideal situation for defining and assessing the baseline scenario for the costs of any of the remediation options. Others are derived from an extensive literature search that addresses the costs of similar technique. The next section will present the results and the discussion that would follow.

5.3 Results

An analysis of costs for the different remediation options are presented in Table 5.3 below. The costs are derived from Table 5.2 which was presented on a unit volume basis (cost/tonne). The analyses give an opportunity to isolate the direct and indirect

costs. The direct costs are incurred for carrying out the remediation activities such as the cost of disposing of the contaminated soil to landfill, cost of re-filling the earth with new soil, landfill tax, the cost of collecting BSG, labour and material costs during remediation activities and soil sampling costs.

The indirect costs are made up of maintenance or organisational costs of carrying out the remediation activities and the costs associated with the delay in developing the land. The costs of delayed development were identified and attributed to each technique. In this study they were based upon the rentable value that will be lost whilst the remediation activities were going on. These indirect costs cut across the six remediation options. The costs for the different remediation options are described in the next sections.

Remediation activities	BR BSG	BRW BSG	NA	LD	SVE	TD
Direct costs						
Disposal cost (Including landfill tax)	-	-	-	£2,040,000		
Re-fill cost (soil)	-			£390,000		
BSG	£85,000.00	-	-	-		
Material and Labour cost	£240,000.00	£360,000	-			
Operating and monitoring	£121,200	£181,800	£444,400	£14,400	£1,000,000	£1,440,000
Indirect costs						
Rental lost	£972,000	£1,458,000	£9,720,000	£648,000	£5,832,000	£1,296,000
Welfare	£3000.00	£4500	-	£2,000.00		
Overall cost	**£1,421,200**	**£2,004,300**	**£10,164,400**	**£3,094,400**	**£6,832,000**	**£2,736,000**

Table 5.3: Results of economic costs including direct and indirect costs of the four chosen remediation options(bioremediation with brewery spent grain-BR BSG, bioremediation without brewery spent grain-BRW BSG, natural attenuation- NA, landfill disposal- LD, in situ soil vapour extraction-SVE and in-situ thermal desorption- TD)

5.3.1 Direct Costs

5.3.1.1 Bioremediation with and without BSG

The total cost of BSG was £85,000.00. This is based on a cost of £38.00/tonne with 2000 tonnes being needed for bioremediation at the case study site in addition to the cost of transportation. Transportation costs of £4.50/tonne were used assuming that the distance from the brewery to the contaminated land site was 30 miles (Ben-Hamed, 2012).

The sampling costs associated with testing the breakdown of the hydrocarbons in

the soil were derived from a cost of £101.00/100 m^3 of soil in addition to personnel costs. The total cost was based on 10,000 m^3 of soil in the contaminated land, which amounts to £20,000.00/month when the site is sampled twice a month. Since bioremediation with BSG has previously been determined to take 6 months and the soil would be sampled twice monthly and bioremediation without BSG takes 9 months the costs are £121,200.00 and £181,800.00 respectively. These costs were grouped under operating and monitoring costs.

Other direct costs (material and labour costs) of bioremediation also include the cost of turning and tilling the soil during the bioremediation process. This was estimated at £1.00/tonne for the case study site. The soil needed turning every two weeks for a period of 6 months for bioremediation with BSG and 9 months for bioremediation without BSG and this amounts to £40,000/month, giving a total of £240,000 and £360,000 for bioremediation with and without BSG respectively.

5.3.1.2 Natural Attenuation

The main direct cost for natural attenuation is the monitoring cost of sampling the soil during the period of attenuation. It costs £101.00/100 m^3 given that there are 10 ha (10,000 m^3 of soil) of land in the study the cost of sampling for one month will be £10,100.00 including personnel costs. The sampling would be carried out on a monthly basis for the first 3 years and quarterly for the remaining 2 years. As the remediation objective for natural attenuation is expected to be achieved in 5 years the total cost of sampling for the period would be £363,600.00 for monitoring, for 3 years on a monthly basis and £80,800.00 for monitoring quarterly, for the remaining 2 years. The total monitoring cost would be £444,400.00.

5.3.1.3 Disposal and re-filling the land

The direct cost of disposing of the contaminated soil to landfill from the case study site was estimated at £102.00/tonne. Since there are 20,000 tonnes of soil for disposal, the cost would be £2,040,000.00. In addition, to re-fill the site with topsoil would cost £19.50/tonne, which amounts to £390,000.00. The total cost fo disposed and replacement of the soil will be £2,430,000.00 given the same 20,000 tonnes of soil that was excavated and disposed to landfill. An equivalent amount was used to re-fill the land. Other direct costs associated with the landfill option are the cost of

labour (foreman or supervisor), this cost has been previously given to be £900.00/week (Table 5.2) and since disposing and re-filling has previously given to be 16 weeks (section 5.2.1. b) the total cost would be £14,400.00.

5.3.1.4 Soil vapour extraction (SVE)

The cost of in situ SVE in the study will adopt from the cost factor of the Defra range for treated soil of more than 5000 m^3. The estimate includes the number of wells, the blower capacity and vacuum level required and the length of time needed to remediate the site. The cost will also take into consideration the off-gas and water treatment that would be extracted prior to disposal. The Defra report was seen to be representative of the average cost, in the remediation sector, as it takes into cognizance different remediation costs from different practitioners (Defra, 2010). In situ SVE costs £100/m^3 when large volumes of soil are remediated (Table 5.2). In this study there is 20,000 tonnes of soil which is equivalent to 10,000 m^3 (Table 5.2) if the cost of £100/m^3 is applied to the volume of soil the final costs will be £1,000,000.

5.3.1.5 Thermal desorption

The cost of thermal desorption used in this study was adopted from a Defra report in which the cost of treating more than 5000 m^3 of soil was estimated to be £144/m^3. This cost includes operation and maintenance costs. This cost was considered as a reliable cost of remediation for thermal desorption in the U.K. because Defra in its report adopted it as their baseline when evaluating the cost of different remediation strategies. If this cost was considered when remediating 20,000 tonnes of soil (10,000 m^3) the cost of thermal desorption in the study would be £1,440,000.

5.3.2 Indirect Costs

5.3.2.1 Bioremediation with and without BSG

The indirect costs associated with bioremediation were calculated based on the period of time to achieve the remediation objective. The rentable value for a 3 bedroom house was previously valued at £540.00/month (Table 5.2) for the location in the case study. If the bioremediation process takes 6 months the rentable value that would have accrued over the period would be £3,240.00 per housing unit and for

300 housing units this will amount to £972,000.00 for bioremediation with BSG and £1,458,000.00 for bioremediation without BSG.

The other indirect cost attributable to bioremediation is the cost of maintaining the site during the period of remediation. For 10 ha of land the monthly cost was previously valued to be £500.00 (Table 5.2). Since bioremediation with BSG remediation objective would be achieved in 6 months it will cost £3,000 for maintaining the site. Whilst bioremediation without BSG will cost £4,500.00 as the time frame for remediation objective to be achieved was previously estimated to be 9 months.

5.3.2.2 Natural attenuation

The indirect costs associated with natural attenuation were calculated based upon the time taken for the breakdown of the hydrocarbons. In this study the time for natural attenuation to attain the remediation targets has been estimated to be 5 years. Leaving the land undeveloped for that period of time would lead to loss of revenue due to the rental value that would have been accrued. The rental value for a house in Sunderland where the project is sited was valued to be £540.00/month (Table 5.2). For a year it will cost £6480.00 and for 5 years it will cost £32400.00. As the housing units are 300 the total amount would be £9,720,000.00.

5.3.2.3 Disposal to Landfill

The indirect costs associated with landfill include the delay of 16 weeks (4 months) that it would take for the soil to be disposed of and the site to be re-filled with new topsoil and subsoil. The rentable value for 4 months was £2160.00 (£540.00/month) and for 300 housing units the amount would be £648,000.00.

There will also be organisation and maintenance costs attributed to landfill, as the disposal and reinstatement exercise will require 4 months to complete. The administrative cost of maintaining the site is £500.00/month (Table 5.2) and for the period of 4 months it would cost £2000.00.

5.3.2.4 Soil vapour Extraction (SVE)

SVE remediation objective has been previously estimated to be completed in 3 years given the default value of 20,000 tonnes of soil (10,000 m^3). The cost of not

developing the land for 3 years would be calculated on the rental value that would have been lost if the land was not developed for housing units. In this study £540.00/month (Table 5.2) was calculated for a housing unit, the cost for 3 years would be £19,440.00 and if 300 housing units are to be built the total cost would be £5,832,000.00.

5.3.2.5 Thermal desorption

The delay cost associated with thermal desorption is the time it takes to achieve the remediation target. This has previously been stated to be 8 months and each housing unit would generate an income of £540.00/month, the total delay cost would amount to £4,320.00 for a housing unit and the total housing units considered in the study were 300 this gives an equivalent amount of £1,296,000.00.

5.4 Sensitivity Analysis

Sensitivity analysis evaluates the various costs associated with each technique if the conditions and certain factors were to change whilst other variables remain constant. In calculating the cost of each technique the cost estimate used in arriving at the result of Table 5.3, was the default cost when 20,000 tonnes of soil was remediated. The sensitivity analysis in the next section will determine the cost of each technique if any of the variables changes and the possible economic costs will be compared to the default cost and will be prorated.

5.4.1 Economic Costs Associated with Varying Soil Quantities

This analysis determines the sensitivity of remediating different tonnages of soil. Table 5.4 shows the analysis of the costs for the different amounts of soil that would be remediated. The total costs in Table 5.4 are derived from the same cost estimate used in arriving at Table 5.3 but different tonnes of soil are used (40,000, 10,000 and 4,000). These costs are made up of direct and indirect costs for the different remediation options. The cost values from varying the soil from 4,000 to 40,000 tonnes were pro-rata to take all variables into consideration.

Qty soil M^3	BR –BSG	BR -WBSG	NA	LD	SVE	TD
20,000	£1,867,400	£2,546,100	£10,608,800	£5,835,000	£7,832,000	£4,176,000
10,000	**£1,421,200**	**£2,004,300**	**£10,164,400**	**£3,080,000**	**£6,832,000**	**£2,736,000**
5,000	£1,198,100	£1,733,400	£9,942,200	£1,377,500	£6,332,000	£2,016,000
2,000	£1,064,240	£1,570,860	£9,808,880	£567,250	£6,632,000	£1,632,000
	BR- BSG = bioremediation plus brewery spent grain BR-WBSG = bioremediation without brewery spent grain NA = natural attenuation LD = landfill SVE = soil vapour extraction TD = thermal desorption Qty = Qunatity					

Table 5.4: Economic costs of different remediation techniques with varying soil quantities the costs were derived from the same estimates used for Table 5.3

The default estimate of 20,000 tonnes (10,000 m^3) of soil and its related costs depicts the typical amount of soil present across various derelict sites in the U.K. (Pers.comm, 2011). The results from Table 5.4 shows that the bioremediation technique has a higher cost when compared to landfill when a lower volume of soil was remediated, in this case 2,000 m^3 (4,000 tonnes) of soil. When comparing the cost of bioremediation with landfill the cost of the former becomes lower as the volume of soil increases for instance when 10,000, 20,000 and 40,000 tonnes of soil were remediated the costs of bioremediation decreases compared to landfill. But the comparison of bioremediation with natural attenuation does not follow this pattern, as regardless of the volume of soil, that needed remediation, the cost of natural attenuation is higher than bioremediation. More so, the cost of bioremediation with BSG and bioremediation without BSG showed that the former were higher given

higher volumes of soil.

Contrary to the cost of bioremediation the costs of SVE was higher irrespective of the volume of soil to be remediated. Thus, the cost of bioremediation with BSG was £1,064,240.00 when 4,000 tonnes (2,000 m^3) of soil was remediated and the cost of SVE was £6,632,000.00. This further increase to £1,867,400.00 on bioremediation when 40,000 tonnes (20,000 m^3) was remediated and SVE cost stands at £7,832,000.00. When comparing the cost of bioremediation with thermal desorption the cost of bioremediation becomes lower as the volume of soil increases for instance when 10,000, 20,000 and 40,000 tonnes of soil were remediated compared to thermal desorption (Table 5.4).

Sensitivity analysis could be used to depict the costs of different remediation techniques and show how variation in input data such as the time scale of the different remediation targets, quantity of soil needing remediation, landfill taxes and remediation strategy can affect the cost of any given remediation method. For example, if the times scale for natural attenuation to achieve the remediation objective is to be extended from 5 years to 10 years or reduced to 2 years the direct and indirect costs would be impacted. Similarly, if landfill tax increased from £38.00/tonne to £100.00/tonne the cost of disposing of the soil to landfill will go up and the technique will be less attractive even when disposing of small amounts of soil. Therefore, carrying out sensitivity analysis allows the input data to be varied and costs to be determined in relation to the remediation goal. In this study the focus is on time scale and conversion into monetary terms for the different remediation options considered in the study.

5.5 Discussion

Economic cost estimation can be difficult, depending on the class of costs under consideration, as it will normally require inputs from various disciplines including accountants, economists and other specialists (HMTG, 2011). Costs of a project are defined according to the different actions undertaken in a geographical unit and the prices, quantities and the values for the total duration of the project (Edward-Jones, 2000).

The cost of a remediation project can be classified as direct and indirect. Direct costs

are activities that can be measured in terms of financial values ascribed to them and can be quantified with some accuracy. These costs include the cost of site investigation, acquiring land, excavation, labour and capital cost and costs of raw materials (Sanders and Vernon, 2009 and EA, 1999) and other relevant costs depending on the type of remediation technique (McEntee, 1991). While indirect costs reflect activities that indirectly bring about costs during the period of remediation, such as the time scale it takes for the remediation goal to be achieved, the cost of maintaining the site during remediation activities and other indirect costs include community disturbance, financial risk, procurement and technology risk (SuRF-UK, 2010).

Categorising remediation costs in this way will enable practitioners to build a safe, durable and economic structure (Tedd, 2001). In appraising any environmental project HM treasury (U.K.) stipulates the indirect cost of a project should be brought into the assessment. Although the costs associated to indirect costs could be difficult to assess but should not be ignored simply because they cannot be easily costed (HMTG, 2011).

Land remediation is becoming a growth sector in the U.K. and long term derelict and vacant brownfield sites form a major proportion of brownfield land in many cities, not only in the U.K. but also in other countries (Dixon *et al*. 2011). There is the need to redevelop these sites as government targets for new homes have been increased (CLG, 2012). The process is mainly driven by cost considerations (Euro Demo, 2006) with substantial amounts of money being spent on redeveloping contaminated land (Taylor and Ramsy, 2005). Estimating the true costs will provide guidance to remediation professionals and enable them to make informed decisions on the choice of remediation strategy. Incorporation of the indirect costs is often problematic for redevelopment projects and hence they may be omitted. Decisions are often based upon costs that do not accurately reflect the true costs of redevelopment and this can often bias decisions against bioremediation technology. Because the direct cost of remediation can be quantified with some degree of accuracy they are often used to justify the choice of adopting a remedial project.

Bioremediation techniques are often associated with long delays because the process is slow and may result in high costs due to the time scale for the remediation

target to be achieved. However, in the study here the inclusion of indirect costs which are associated with the delayed development of the land may make remediation options such as bioremediation more feasible and comparable.

In terms of indirect costs Mohamed and Irani (2002) identified indirect costs to include learning costs, costs of resistance and costs of redefining roles, and time costs amongst others when evaluating direct and indirect costs that are involved in information systems. They stated the time spent in training staff or undergoing management training are not often reflected in the organisation accounting book unless the organisation obtains a time recording system, where the indirect cost of time spent in training would be accounted for as information system costs. Rather the organisation would regard the indirect costs as hidden costs in addition to other costs such as time spent in reading manuals, self-help activities, and informal job training, which are not traceable.

In another study Love (2002), undertook a study on the indirect consequences (financial cost) of re-work in construction projects and estimated the cost of rectifying the defects of work left by sub-contractors and ascribes financial value to waiting times (3 hour @ $35/hour) and loss of productivity as a result of the delay in executing the job (4 hour @ $ 35/hour). It was found that the indirect cost was six times higher than the direct costs.

In land remediation the evaluation of indirect costs associated with remediation can be measured using different economic models. Messer *et al*. (2000) studied the long term impacts of delayed clean-up on property values in communities neighbouring prominent Superfund sites in the U.S. The research studied the sales prices of about 34,000 homes near sites of contamination in three metropolitan areas for 30 years and found a correlation between delayed clean-up and loss of property values.

In the study reported here a case study site was developed. It is typical of brownfield sites in the U.K. and this chapter evaluated the economic costs associated with the remediation of 10,000 m^3 of soil using a range of techniques including bioremediation with BSG. The addition of the indirect costs makes bioremediation comparable to other remediation options however this is unlikely to happen in practice. Figure 5.2 below shows the total cost of remediating 10,000 m^3 using the different remediation options evaluated in the study.

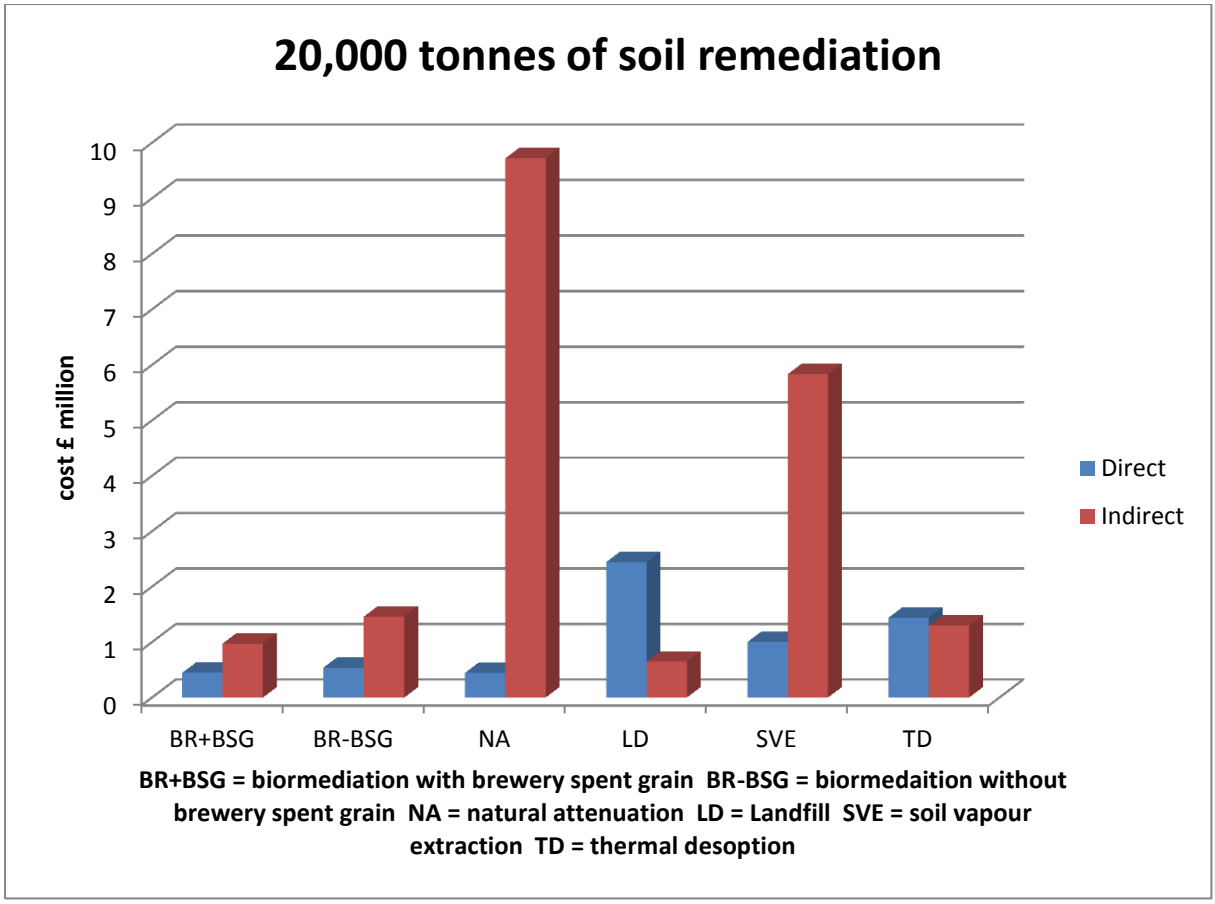

Figure 5.2 Total costs for remediating 10,000 m^3 of diesel contaminated soil using the technologies assessed in this study.

Figure 5.2 is made of direct and indirect costs for the different remediation techniques used in this study. The direct costs are the actual costs that have market values and these costs can be easily monetised because they can be quantified by their activities and services rendered. The results show that bioremediation with BSG has a lower cost than bioremediation without BSG this is due to the costs associated with delayed development which are greater for bioremediation without spent grain than bioremediation with spent grain.

Ascribing values to indirect costs, such as the time delay in carrying out the remediation work, could be compared with the monetary values of the alternative options, such as the benefit stream of an investment project. In this case, it was attributed to rental values that would have been accrued during the period of remediation. The time of not putting the land into use can be comparable to the amount of money that would be lost because of the delay in remediating the land.

The results from this study (Figure 5.3) showed that natural attenuation was less

attractive when the indirect cost was considered. This is because of the delay in remediating the soil and the revenue that would have been lost in that timeframe. In a similar pattern when comparing the landfill option to bioremediation with BSG the landfill method required a shorter time (less indirect costs) to dispose of the contaminated soil, yet it was less attractive to bioremediation in terms of total costs. The high cost of landfill disposal can be attributed to include the capacity of the landfill sites to accept contaminated materials, as very few landfill sites are designated to receive hazardous waste in the U.K., there are also the costs of diesel, distance from the landfill and the current landfill tax.

The interactions of these factors have a substantial impact on overall unit rates of the soil disposed to landfill. However, if any of these factors changes to favoure landfill the costs may become comparable to bioremediation.

The technique of SVE and thermal desorption have higher direct and indirect costs (delayed) compared to bioremediation. The high costs are attributed to the energy demand of the technology, which is high in the U.K. However, if this is compared in another country, for instance the U.S. where energy costs are low, these techniques could be comparable and this could be the reason why thermal desorption and SVE are widely used in remediating hydrocarbon soils in the U.S. (Williams and Brankley, 2006). However, SVE is more effective in removing organic contaminants with vapour pressure greater than 0.5 mm mercury and this includes chlorinated solvents, such as trichloroethene (TCE), gasoline and many other toxic compounds found in jet fuel (U.S. AFERP, 2001). In order to use the technique to remediate diesel soil air injection will need to be promoted but some components of diesel may be difficult to remove (U.S. EPA, 1991) and the time frame for removing these diesel components will be significantly extended. This limitation may stall the use of SVE and make it less attractive to remove diesel compounds from soil due to the indirect costs that would be incurred.

High energy usage by thermal desorption may result in economic activities that would lead to greenhouse gas emissions, which is likely to have consequences far into the future. The U.K. government has set up effective ways of delivering the required emissions to meet carbon budgets set under the Climate Change Act. Using techniques such as thermal desorption in the U.K. will incur a carbon tax if proper

abatement technology is not used and this may add to the operating cost of this technology.

To ensure the cost of carbon is better integrated into remediation costs and to reduce overhead costs renewable energy is a key to low carbon energy in the future as this will help to reduce GHG emissions and to diversify alternative energy sources. Hence bioremediation will become attractive as the technology does not require high energy demand.

However, categorising costs in this manner can aid sensitivity analysis in that a cost that is fixed relative to one factor may change with another. SuRF-UK, (2010) suggest that sensitivity analysis should be considered as an element of sustainability assessment when evaluating remediation projects in order to accommodate the variation in input data and how assumption can influence the overall outcome of an assessment. The next section describes the sensitivity analysis of various remediation options considered in the study.

5.6 Sensitivity analysis

The analysis focuses on how conclusions on the total costs depend on certain assumptions used to arrive at the conclusions. In other words the analysis tests whether the variables used hold a wider confidence margin that accounts for the differences in measuring various costs. These analyses are inherent subjective values of the assessors, as it is possible that different assessors presented with the same scenario and information may arrive at different assessments or optimumal solution. Therefore, the purpose of this analysis is to underpin the need for assessors to understand the variability of their data input as any significant changes in certain input may alter the outcome of the assessment. Hence in the present study sensitivity analyses were carried out to take into account when different quantities of soil were remediated and various costs associated with each technique if the conditions and certain factors change while other variables remain constants.

5.6.1 Sensitivity analysis for comparing the costs of 40,000, 10,000 and 4,000 tonnes for the six different remediation options

The sensitivity analysis was derived from the same costs used to remediate 20,000 tonnes of soil in table 5.5.1 and the direct and indirect costs were pro-rata. This

analysis showed costs when 40,000, 10,000 and 4,000 tonnes of contaminated soil is to be remediated. Fig. 5.3 below compared these costs with the different remediation options.

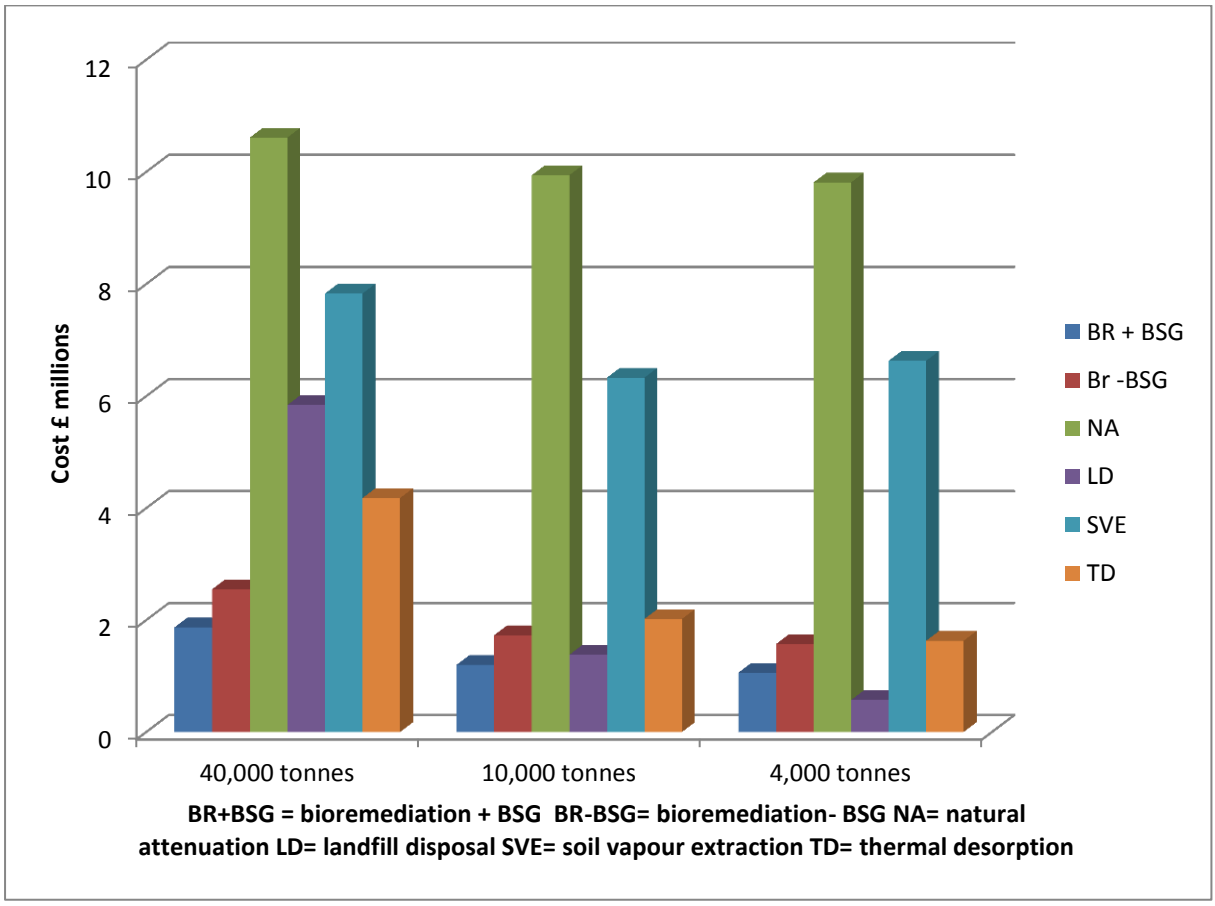

Fig.5.3 conomic costs of remediating 40,000, 10,000 and 4,000 tonnes of soil using the same estimate for Table 5.3

Figure 5.3 above shows how the cost is sensitive to the quantity of soil to be remediated. When 40,000 tonnes (20,000 m^3) soils is to be bio remediated the cost rises, but with the lower amount of soil to be remediated the cost reduces. The bioremediation treatment without BSG follows a similar pattern as the volume of soil reduces the cost decreases.

When natural attenuation is considered the increase or decrease in the volume of soil makes little or no changes to the costs of remediation, the reason being that the technique takes the same time frame for soil to be remediated irrespective of the volume of soil to be cleaned and the indirect cost that constitutes the bulk of the total cost remains unchanged as indicated in Figure 5.3.

The consideration of the landfill option in the sensitivity analysis shows that the cost of remediation is a function of the amount of soil to be remediated. There is a huge decrease in the cost of landfill as the volume of soil reduces, the reason being that the cost of the landfill tax decreases and the cost of disposing of the soil also reduces and these have an effect on the direct and indirect cost of the landfill option.

The results from Figure 5.3 shows there is a sharp decrease in the costs of thermal desorption as the volume of soil to be remediated decreases. The reason could be attributed to lower energy cost. However, if thermal desorption can make use of current technology such as a heat exchangers which can recover approximately 80% of heat energy from the organic pollutant in the contaminated soil (URL7) and use the energy as alternative power source, the operating costs will be reduced. The use of this technology may make thermal desorption more comparable with bioremediation especially when a large volume of soil is involved and the cost of the carbon tax could be eliminated. However, this is not the case for SVE technique as there are little or no changes as the quantity of soil varies in relation to the total cost. This insensitivity of the cost in relation to the quantity could be attributed to the indirect cost of delay and high fixed cost that is associated with the remediation option.

Bioremediation becomes more promising and attractive to adopt when the volume of soil is high such as 10,000 m^3 (20,000 tonnes) and 20,000 m^3 (40,000 tonnes) Landfill option becomes attractive as the volume of soil decreases from 5,000 m^3 to 2,000 m^3 (4,000 tonnes) and comparable to other remediation options including bioremediation and this could be the possible reason why most remediation practitioners opt for landfill when the volume of soil is small.

The adoption of other techniques such as bioremediation without BSG, thermal desorption and SVE in the study does not lead to a reduction in costs compared to bioremediation irrespective of the volume of soil to be remediated as these techniques are still characterised with high direct and indirect costs.

Consequently, bioremediation could be viewed as a good option and often not used by remediation practitioners because of economics and the need to develop the land on time (PWTB, 1999). Often in the U.K. landfill is considered the preferred option (CL:AIRE, 2007). However, the cost associated with the delay is not easily quantified

in monetary terms and is often not considered in the selection of remedial options. But expressing this delay in terms of the relevant opportunity cost of not using the land during remediation activities and monetising it allows for the full cost of remediation to be evaluated.

5.7. Conclusions

Remediation costs should normally be extended to cover the period of the useful lifetime of the project. The costs to be considered should include direct and indirect costs. In this study, using direct and indirect costs, the cheapest option was bioremediation with BSG with natural attenuation being the most expensive option.

However, if the direct costs are used the cheapest would be natural attenuation and the most expensive will be thermal desorption. Landfill, an option currently most often used for this type of site in the U.K., showed that the evaluation of the direct and indirect cost is in the middle of the cost hierarchy highlighted.

More so, in the study here sensitivity analysis is used to demonstrate how sensitive remediation costs could be in practice, if certain conditions change. The sensitivity analysis also shows that remediation costs are sensitive to certain conditions and could be a function of the purpose of the site, technology used and duration of the remediation works. The costs used in the present study were obtained from a remediation practitioner (Pers.comm, 2011) and others were derived from a literature search such as past remediation projects, company reports and Defra reports, which is a repository of all remediation, work in the U.K. The feasibility of using these costs was checked with the remediation practitioner (Pers.comm, 2011) in line with current inflation levels before being used. But economic costs are only one aspect of sustainable development hence the next chapter of this thesis goes on to evaluate the environmental costs of the proposed options.

Chapter 6

ENVIRONMENTAL IMPACTS OF THE DIFFERENT REMEDIATION OPTIONS ADOPTED IN THE STUDY

6.1 Introduction

This chapter aims to determine the environmental impacts of the six different remediation options considered in the study in relation to air, water and land pollution. Soil respiration measured in the laboratory was used to calculate the amount of CO_2 emitted during bioremediation of diesel contaminated soil with/without the addition of BSG. In addition, data collection was used to derive other sources of emissions for the different remediation methods considered in the study.

The remediation options considered in the study were quantitatively and qualitatively evaluated in relation to the three media (air, water and land) to determine, which remedial option is least environmentally sustainable. The next sections describe the methods used to determine the environmental impacts of various remediation options, including the determination of CO_2, data collection, emission factors and their justification, results, discussion and conclusion.

6.1.1 Environmental impacts and land contamination

The post industrial revolution saw a decline in environmental quality especially air, water, land and the depletion of natural resources (Edward-Jones *et al*. 2000). During the past two decades, public concern regarding environmental issues has risen in line with awareness of increasing pollution levels, loss of natural habitat and the effect of environmental degradation upon human health and well-being (Bonnieux and Rainelli, 1999). Therefore, members of the industrial society are re-thinking how behaviour, reliance on technology, and consumption of energy are impacting on the environment.

Contaminated land remediation experts are also aware of the concerns and the impacts of remediation activities on both the environment and in particular on global climate change (Ellis and Hardley, 2009). Petroleum hydrocarbons are common environmental contaminants and are ubiquitous in both the terrestrial and aquatic environment (Muijs and Jonker, 2009). In addition there are a range of technologies

used in their clean-up, with the different technologies having differing impacts upon the environment.

Previous chapters of this thesis have evaluated several methods in terms of their effectiveness and their economic viability. The range of methods evaluated in the study reported here can be divided into traditional engineered methods such as landfill, natural attenuation and SVE and newer process-based methods such as bioremediation and thermal desorption. The results reported in previous chapters indicate that each of these methods differs in terms of cost, effectiveness and the time scale required for clean-up. However, these remedial options may have associated environmental impacts and health risks to both workers at the site and the nearest neighbours.

The traditional engineering methods such as landfill or offsite disposal simply move the contaminants to a different location (Mansfield and Mohan, 2002). Moving contamination around the country for disposal in a landfill creates intrusion through vehicle movements and may also increase the potential risk to human health and the local environment through traffic accidents and exposure to dust (EA, 2004). The Royal Commission for Environmental Pollution (RCEP) described engineered methods as environmentally unsatisfactory compared to process based techniques in that the process based techniques remove the contaminants from the soil and break them down into harmless substances including CO_2 and water (RCEP 1996). However, these techniques are less familiar within the remediation industry as they frequently lack a comprehensive history of usage (Mansfield and Moohan, 2002) and may not be as environmentally friendly as initially thought.

In order to achieve sustainable remediation practices the environmental impacts of bringing about the remediation process should be minimal and measurabl (CL: AIRE, 2007). Net environmental benefits have recently been identified as one of the main drivers of remediation and if fully optimised are expected to result in effective clean-ups that maximize the environmental benefit whilst protecting human health and the environment (Ellis and Hardley, 2009). Additionally, the environmental impacts of implementing the remediation process should not be more than the impacts of leaving the land untreated (CL: AIRE, 2007).

In the U.K. environmental impacts that are associated with remediation activities

have been identified and are expected to be measured during remediation activities according to SuRF-UK (2010). The template used by SuRF-UK in the measurement of environmental impacts includes the following indicators: impacts to air, water, soil and ecology, use of natural resources, waste generation (effluent discharge), emissions of greenhouse gases, haulage distance, energy consumption, climate change, biodiversity, water consumption, dust monitoring, vehicle movement (road wear, road traffic accident and emissions) and carbon footprints. In the review of Jackson and Roberts (2000) they asserted that in a situation where a long list of indicators are provided when measuring the sustainability of projects, a shortlist appropriate to the intended goal based on the desired characteristic for such indicators should be chosen.

The study reported here seeks to determine the environmental impacts of bioremediation with and without BSG. In order to achieve this aim the following four techniques were evaluated in the study.

- The impacts of bioremediation and natural attenuation will be evaluated by determining the volumes of CO_2 released using respirometry.
- Selected key performance indicators considered significant to the impacts of the remediation work will be evaluated in line with the activities of the remediation techniques.
- The environmental impacts on air, water and land resulting from the different remediation activities will be evaluated through the collection of secondary data.
- The information obtained will be used to cost the environmental impacts of bioremediation with and without BSG and the results will be used to compare other remedial options such as landfill, natural attenuation, SVE and thermal desorption.

6.2 Methods

An evaluation of the methodology developed to determine the environmental impacts of remediation methods has been based on the case study site developed in Chapter 5. The scenario of the case study is a typical conceptual contaminated land model in

the U.K. (Pers.comms, 2011).

In the chapter reported here environmental impacts were determined for air, land and water. Two techniques have been used to determine environmental impacts:

- Practical methods – the measurements of CO_2 release by respirometry.
- Secondary data collection.

Both methods will be described in the following section.

6.2.1. Practical methods

The measurement of CO_2 as a result of soil respiration is a means of measuring carbon emissions and hence one of the environmental costs for biological remediation of soils. Measuring CO_2 emissions during the bioremediation process, in the field, is problematic as it is difficult to capture the CO_2 released accurately. However, in the laboratory, using a common chamber method such as a closed flask, where there is no air-circulation, the CO_2 efflux can be accurately measured by chemical titration after it has been absorbed into a solution.

The piece of work reported here seeks to determine emissions of CO_2 during the bioremediation process. The information that was obtained can then be used to cost the environmental impact, in terms of greenhouse gas emissions, for bioremediation with and without BSG. The materials used in the study have already been described in sections 3.2.1, 3.2.2 and 3.2.3.

Table 6.1 below shows the composition of the treatments and how they were applied in this study.

Treatment	% of diesel in the soil	Grams of soil + diesel	BSG % added in grams	Number of bottles
1	0%	2000g	-	3
2	5%	2000g	-	3
3	5%	2000g	200g (10%)	3
4	5%	2000g	400g (20%)	3

Table 6.1 - Composition of the different treatments evaluated: soil only (control), soil plus diesel, soil plus diesel plus 10% BSG and soil plus diesel plus 20% BSG.

In preparing the experiment the same protocol used in section 3.2.4 was adopted and applied. Each treatment consisted of 2 kg of mixed soil treated as detailed in Table 6.1 above. For each treatment the soil was transferred into a bottle of 2.5 litre capacity with three replicates being used per treatment. Two holes were made in each bottle and tube connectors were attached. The CO_2 in the bottle was allowed to build up as a result of the hydrocarbon degradation and measurements were taken every two weeks.

Figure 6.1 shows the construction of the experiment and the soil in the 2.5 litre bottles being incubated in the oven at a temperature of 28-30^{0}C.

Figure 6.1- The 2.5 litre bottles containing the treatments: soil only, soil plus diesel, soil plus diesel plus BSG (10%) and soil plus diesel plus BSG (20%)

The following method was adopted to determine the soil respiration at the end of every two weeks.

6.2.2 Measurement of CO_2 and determination of soil respiration

At the end of the two week period CO_2 was captured from the bottles using an air pump. It was collected by bubbling the air plus the CO_2 through a solution of sodium hydroxide. The procedure and method used in collecting the CO_2 are broken down into two sections below:

1. The CO_2 trapped in the bottles was captured and transferred into a 250 cms^3 Erlenmeyer flask. To do this one of the tubes in the bottle was inserted into an air pump and the other tube was connected into the top of the 250 cms^3 Erlenmeyer flask, which has a stopper tube connected to it. Then another tube was connected from the side of Erlenmeyer flask to an arm test tube. The arm test tube has an opening at the side of it. Figure 6.2 illustrates a conceptual layout of the construction and how the CO_2 was trapped and contained.

Figure 6.2: Materials used to trap CO_2 from the contaminated soil with diesel in the laboratory including the air pump to transfer the CO_2 into the Erlenmeyer flask and the test tube used.

The trapped CO_2 in the bottle was pumped slowly into the sealed Erlenmeyer flask, which contains about 50 cms^3 (Approximately 1M) of sodium hydroxide. The pumping process took about 24 hours. The containment of the CO_2 in such a closed compartment and its transfer to a sealed apparatus allows the sodium hydroxide to absorb the CO_2. More so, the purpose of the side arm test tube was to capture additional CO_2 in that if the CO_2 in the Erlenmeyer flask does not mix with the sodium hydroxide the escaping CO_2 would be trapped in the side arm test tube.

2. Prior to undertaking the titration the sodium hydroxide from the Erlenmeyer flask and that from the side arm test tube were mixed in a 250 cm^3 Erlenmeyer flask. The excess sodium hydroxide and sodium carbonate are then titrated as described by Crossno and Kalbus (1996) as follows:

(a) Each solution was titrated with 1M HCI using phenolphthalein as an indicator.

(b) The volume used was recorded for the first end point.

(c) 4 drops of methyl orange were added and the titration was continued until an

orange colour was obtained.

(d) The volume used to the second endpoint was recorded and the amount of CO_2 derived from the soil calculated.

The equation used to obtain the mass of CO_2 (g) was:

Volume of titrant (L) * molarity of standard acid * molecular weight of CO_2

Where

Volume of titrant = 1^{st} to 2^{nd} endpoint

Molarity of standard acid = 1M HCl.

In order to arrive at the actual CO_2 (g) the results of the control soil, which was a treatment without any amendment or without any addition of diesel were deducted either from the treatment with BSG or treatment without BSG. The average CO_2 released from the 100 g of diesel added to the 2 kg of soil during bioremediation recorded on Day 14, 28, 42, 56, 77, 98 and 129 days are reported in Tables 6.2 to 6.8.

Treatments / types	1 Volume of titrant (L)	2 molarity of standard acid	3 molecular weight of CO_2	4 Control soil result (g)	5 Results (g) CO_2
Bioremediation(10% BSG)	27/1000	1	44.01	0.308	0.88
Bioremediation 20% BSG	30.5/1000	1	44.01	0.308	1.03
Bioremediation (without BSG)	24.40/1000	1	44.01	0.308	0.77
Control soil	7/1000	1	44.01	0.308	-

Table 6.2: CO_2 released after 14 days bioremediation with/without BSG

Treatments	1	2	3	4	5
	Volume of titrant (L)	molarity of standard acid	molecular weight of CO_2	Control soil result (g)	Results (g) CO_2
Bioremediation (10% BSG)	14.88/1000	1	44.01	0.118	0.54
Bioremediation (20% BSG)	15.77/1000	1	44.01	0.118	0.58
Bioremediation (without BSG)	8.83/1000	1	44.01	0.118	0.271
Control soil	2.67/1000	1	44.01	0.118	-

Table 6.3: CO_2 released after 28 days bioremediation with/without BSG.

Day 42 CO_2 emitted by four types

Treatments types	1	2	3	4	5
	Volume of titrant (L)	molarity of standard acid	molecular weight of CO_2	Control soil result (g)	Results (g) CO_2
Bioremediation 10% BSG	10/1000	1	44.01	0.0206	0.23
Bioremediation 20% BSG	12.33/1000	1	44.01	0.0206	0.34
Bioremediation (without BSG)	9.33/1000	1	44.01	0.0206	0.21
Control soil	7/1000	1	44.01	0.0206	-

Table 6.4: CO_2 released after 42 days bioremediation with/without BSG.

Treatments	1	2	3	4	5
	Volume of titrant (L)	molarity of standard acid	molecular weight of CO_2	Control soil result (g)	Results (g) CO_2
Bioremediation (10% BSG)	5.33/1000	1	44.01	0.088	0.14
Bioremediation (20% BSG)	6.67/1000	1	44.01	0.088	0.21
Bioremediation (without BSG)	4.9/1000	1	44.01	0.088	0.13
Control soil	2/1000	1	44.01	0.088	-

Table 6.5: CO_2 released after 56 days bioremediation with/without BSG.

Treatments types	1	2	3	4	5
	Volume of titrant (L)	molarity of standard acid	molecular weight of CO_2	Control soil result (g)	Results (g) CO_2
Bioremediation 10% BSG	4.33/1000	1	44.01	0.076	0.12
Bioremediation 20% BSG	5.68/1000	1	44.01	0.076	0.17
Bioremediation (without BSG)	5.67/1000	1	44.01	0.076	0.17
Control soil	1.73/1000	1	44.01	0.076	-

Table 6.6: CO_2 released after 77 days bioremediation with/without BSG

Treatments / types	1 Volume of titrant (L)	2 molarity of standard acid	3 molecular weight of CO_2	4 Control soil result (g)	5 Results (g) CO_2
Bioremediation 10% BSG	2.67/1000	1	44.01	0.038	0.080
Bioremediation 20% BSG	4.66/1000	1	44.01	0.038	0.17
Bioremediation (without BSG)	2.67/1000	1	44.01	0.038	0.080
Control soil	0.87/1000	1	44.01	0.038	-

Table 6.7: CO_2 released after 98 days bioremediation with/without BSG

Treatments / types	1 Volume of titrant (L)	2 molarity of standard acid	3 molecular weight of CO_2	4 Control soil result (g)	5 Results (g) CO_2
Bioremediation(10% BSG)	4.66/1000	1	44.01	0.015	0.19
Bioremediation 20% BSG	11.55/1000	1	44.01	0.015	0.49
Bioremediation (without BSG)	3.9/1000	1	44.01	0.015	0.16
Control soil	0.33/1000	1	44.01	0.015	-

Table 6.8: CO_2 released after 129 days bioremediation with/without BSG

Sample day	Soil + Diesel CO_2 (g)	Soil + Diesel + 10% BSG CO_2 (g)	Soil + Diesel + 20% BSG CO_2 (g)
Day 14	0.77	0.88	1.01
Day 28	0.27	0.54	0.58
Day 42	0.21	0.23	0.34
Day 56	0.13	0.14	0.21
Day 77	0.17	0.12	0.17
Day 98	0.08	0.08	0.17
Day 129	0.16	0.19	0.49
Total	**1.79**	**2.18**	**2.97**

Table 6.9: CO_2 measured on days 14, 28, 42, 56, 77, 98 and 129 for soil plus diesel, soil plus diesel plus BSG (10%) and soil plus diesel plus BSG (20%).

Table 6.9 provides a summary of the four treatment types of CO_2 released when 100 g of diesel was added to (2 kg) of soil during bioremediation activities recorded on Day 14, 28, 42, 56, 77, 98 and 129 days.

6.3 Data collection

The estimation of the CO_2 emitted by bioremediation with and without BSG was based on the laboratory experiment conducted in this study. The results showed that using BSG resulted in more CO_2 being emitted than in the absence of BSG. The amount of CO_2 obtained from the study was a useful means of ascribing data to the environmental impacts of bioremediation. In order to assign values for other remediation techniques information was sourced from a variety of sources such as a literature search, including professional journals and publications, this information was then applied to the hypothetical case study site.

Actual field data on environmental footprints for the full range of remediation techniques used for contaminated land sites are sparse, in that it is impractical to obtain environmental data that would be representative of all project applications, site types, and conditions. Whilst some case study data are available for sites where a specific remediation technique has been used the scenario may be difficult to apply to other remediation methods or sites. Therefore, field data relevant to the remediation scenario investigated here has been used, when available, to verify some of the assumptions used in the analysis.

Data has been collected for land, air and water but values will only be assigned to air, due to the limited availability of data for water and land, environmental emissions to these media will be qualitatively assessed in relation to the hypothetical site under study.

Key assumptions and inputs such as energy and emission conversion factors and average energy used will be considered in the analysis. For example, electricity will be used as a default factor for all energy consumption for the six different remediation methods in the study and Heavy Goods Vehicle (HGVs) will be used to deliver material and waste in the study because of the large volume of soil that was involved. In addition, it is assumed that the six remediation options under consideration will achieve more than 90 % of the remediation objective and they will have the same level of efficiency in terms of removing the diesel contaminants from the soil.

In this study the values for CH_4 and N_2O are presented as CO_2 equivalent (CO_2e) using a global warming potential factor, consistent with reporting under the Kyoto Protocol and the second assessment report of the Intergovernmental Panel on Climate Change (IPCC). The emission of CO_2 and other gases such as CH_4 and N_2O are potential greenhouse gases (GHGs) and they play major roles in climate change (IPCC, 2007). Each gas has a global warming potential measured as the ratio of heat trapped by one metric tonne of gas to that of one tonne of CO_2 over a specific time period (TRUCOST, 2012) usually 100 years (Colpan *et al.,* 2009). The emissions of these GHGs multiplied by their Global Warming Potential (GWP) are used to calculate the equivalent level of CO_2e emissions (Colpan *et al*. 2009).

The conversion factors allow activities data such as litres of fuel used, number of

miles driven, tonnes of waste sent to landfill to be converted into kilograms of CO_2 equivalent (Defra/DECCs, 2011). Therefore, CO_2e is a term to describe the different greenhouse gases in a common unit (Brander and Davis, 2012). The analysis used in the study is structured so that data that is universally accepted can be used and incorporated subject to adjustment in the units of measurement. The following section shows the spread sheet including the inputs and assumption data used in the analysis.

6.3.1 Emission factors and their Justification

No.	*Data*	*Factors*	Sources/references
1	**Emission factors g per vehicle km**		
2	CH_4 at 0% and 100%	0.36	Defra/DECC GHG conversion factor, 2011
3	N_2O at 0% and 100%	7.75	
4	CO_2 at 0%	671.5	
5	CO_2 at 100%	863.4	
6	Kg/CO_2e/Litre emitted by tractor	2.66	
	Other pollutants		
7	Nitrox Oxide (NOx)	3.870	NAEI, 2012 - http://naei.defra.gov.uk/data/ef-transport
8	Particular Matter (PM_{10})	0.072	
9	Particular Matter ($PM_{2.5}$)	0.068	
10	Carbon monoxide (CO)	0.786	
11	Volatile organic compound (VOC	0.139	
12	Ammonia (NH_3)	0.003	
13	Sulphur Dioxide (SO_2)	0.003	NAEI,2012- http://naei.defra.gov.uk

Table 6.10: Data and sources of information used to derive the emission factors used for environmental impacts presented in this chapter

The factors presented in table 6.10 were derived from the conversion factors presented in (Defra/DECC, 2011). These emission factors are based on g per vehicle km travel. They are used to calculate the g of pollutant concentration in the air. In the U.K. emissions for key air pollutants such as NOx, PM_{10}, $PM_{2.5}$, NMVOC, and CO are calculated by using speed related activity and vehicle flows on the road network. The exceptions are CO_2, CH_4 and N_2O which uses fuel consumption as a proxy for the distribution of emissions. Emission estimates in the UK are calculated by applying an emission factor to an appropriate activity as indicated below:

Emission = Factor x Activity

The emission factors are based on experimental measurements of emissions from in-service vehicles of different types driven under a test cycle with different average speeds (NAEI, 2006). Thus, pollutant concentrations in the air can be measured through a vehicle driven cycle by using road traffic data, vehicle kilometres travelled for different vehicle types and different road classifications on the U.K road network.

Emission factors are normally obtained from measurements on a number of sources representatives of a particular sector (NAEI, 2008). For example emission factors for vehicles pollutants are compiled by National Atmospheric Emission Inventory (NAEI) using fuel consumption and emissions factors derived from the database held by the Transport Research Laboratory (TRL) measured over different test cycles (NAEI, 2008). While activities data are derived from Government statistical sources such as U.K. Energy statistics, Transport Statistics Great Britain and others such as U.K. Petroleum Industries Association (UKPIA), which provide data on the sulphur content of fuels (NAEI, 2008).

In the study here all greenhouse gas emissions have been converted into carbon dioxide equivalent (CO_2e) and it will be reflected in metric tonnes which are used in reporting emissions under the Kyoto protocol. The CO_2e for CO_2, CH_4 and N_2O for Heavy Goods vehicles such as tractors have already had CO_2e incorporated in the emissions factors presented in Table 6.10 and as such need not be calculated separately. However, in the assessment of a particular remediation activity where CO_2e has not been reported, the conversion factors and their global warming potential will need to be applied using the values in Table 6.10.1.

No.	Data	Values	Sources/references
1	CO_2 to CO_{2e}	1	DEFRA/DECC, 2011 GHG conversion factor
2	CH_4 to CO_2e	21	
3	N_2O to CO_2e	310	
4	CO_2 from electricity per kWh (1.37 ibs) equivalent in kg	0.6214	U.S. EIA, 2005 and Electricity power annual, 2005
	Electricity consumed by SVE component over 3 years		
5	Electricity consumption for Vacuum blower measured kWh	108,000	U.S. EPA, 2010
6	Electricity consumption for Off-gas treatment facility measured kWh	90,000	
7	Electricity consumption for data monitoring and processing measured kWh	33,000	
8	Electricity consumption for above ground treatment structure measured kWh	1,800	
	Total electricity consumed kWh	**232,800**	
	Electricity consumed by thermal desorption with a year		
9	M^3 of soil will generate electricity measured kWh	342	Baker, 2011
11	10,000 m^3 of soil will generate electricity measures kWh	**3,420,000**	
	Fuel usage		
12	For 9.7 miles amount of litres of fuel used for HGVs in U.K	4.54609	Department for transport and statistics, 2010
13	14 excavating tractor uses litres of diesel	70	Sirius, 2010
14	Litres of fuel used for bioremediation with BSG	910	Appendix lll
15	Litres of fuel used for bioremediation without BSG	1330	Appendix lll
	Distance of site location		
16	Estimated distance of the site in Sunderland To Hartlepool measured in km	48.28	Pers.comm, 2012- meeting

Table 6.10.1: Showing data and sources of information used to derive the values used for environmental impacts presented in this chapter

These values were used for calculating the CO_2e for bioremediation with and without BSG, natural attenuation, landfill disposal, SVE and thermal desorption. The values use for the calculation of the various emissions is presented in Table 6.10.1 above.

The values for CO_2e used in the study were derived from conversion factors presented by Defra/DECC (2011). These are key pollutants that affect climate change or lead global warming. Other CO_2 calculations were from the use of electricity and in the U.S. it was measured as 1.37 ibs/kWh, which is equivalent of 0.6214 kg/ kWh. In the study reported here the same value was used to calculate electricity usage for SVE and thermal desorption. The electricity consumption was based on components (machines) used in treating the volatile organic compounds or the vapour gases. For SVE it was calculated for 3 years and for thermal desorption it was based on m^3 of soil per kWh (U.S. EPA, 2010a and Baker *et al*. 2011).

In the study reported here heavy goods vehicles (HGVs) were used in tipping, transporting wastes and materials from the contaminated site to the disposable location, they were also used in excavating and turning of the soil during the bioremediation process. To derive the emission for a particular pollutant the emission conversion factor is applied to the distance covered for that journey (NAEI, 2008). The NAEI is the main repository of U.K. data on all types of atmospheric emission from all sources (NAEI, 2012) and the activity data are obtained from the Department of Transport (DFT). In order to derive the activities that lead to the emissions used in the study the distance covered from Sunderland to Hartlepool was estimated to be 48.28 km (Pers.comm, 2011) and 4.54609 litres of fuel are expected to be used to cover 9.7 miles (Dft, 2010). The heavy machinery used in excavating and turning the soil is expected to consume 70 litres of fuel daily this value was used to derive the amount of fuel used for bioremediation with and without BSG (Appendix 111).

As part of the commitments to the Convention on Long-Range Trans boundary Air Pollution (CLRTAP), countries are mandated to submit emission projections for NOx, SOx, NMVOC and NH_3 under the Gothenburg Protocol (NAEI, 2012). These pollutants are also covered under the Directive 201/81/EC of the European Parliament and the Council on National Emissions Ceilings (NECD) and some of these emissions are covered under the UN Framework Convention on Climate Change (UNFCCC) including carbon dioxide, nitrous oxide, methane,

hydroflourocarbons, perfluorocarbons and sulphur hexafluoride which are reported as part of the U.K. inventory. These calculations are shown in the results section below:

6.4. Result

6.4.1. Air pollution

Impacts	BR BSG T CO_2e	BR WBSG T CO_2e	NA Kg T CO_2e	LD Kg T CO_2e	SVE Kg T CO_2e	TD Kg T CO_2e
Process emissions						
CO_2	0.594	0.358	0.358	0.358	-	-
CH_4	0.091	-	-	1.827	-	-
N_2O	28.998	-	-	579.964	-	-
Activities emissions						
CO_2	11.684	3.538	-	185.262	144.66	2,125.19
Total TCO2e	**41.367**	**3.896**	**0.358**	**767.411**	**144.66**	**2,125.19**
Conventional emissions	BR BSG kg	BR WBSG kg	NA Kg	LD Kg	SVE Kg	TD Kg
NOx	46.71	-	-	934.22	-	-
PM_{10}	0.87	-	-	0.93	-	-
$PM_{2.5}$	0.82	-	-	17.38	-	-
CO	9.49	-	-	16.42	-	-
VOCs	1.68	-	-	189.74	-	-
Ammonia (NH_3)	0.04	-	-	0.72	-	-
Sulphur Dioxide (SO_2)	0.04	-	-	0.72	-	-

Where = BR = bioremediation LD = landfill disposal NA = Natural attenuation SVE = soil vapour extraction TD = Thermal desorption T CO2e= Metric tonnes carbon dioxide equivalent

Table 6.11: summarises the pollutants value of emission from remediation process and activities for the different remediation options considered in the study.

Table 6.11 above provides a summary of the CO_2e and other pollutants considered in the study. As can be seen from the results the CO_2, CH_4 and N_2O and other pollutants such as NOx, PM_{10}, $PM_{2.5}$, CO, VOC, NH_3 and SO_2 were calculated from

remediation processes and activities such as transportation and energy. The detailed calculations of each remediation technique are described in the next section.

6.4.2 Bioremediation emissions

The total volume of CO_2 evolved from the bioremediation process with BSG was previously calculated to be 2.97 g and without BSG was 1.79 g when the soil volume was 2 kg with 5% diesel contaminants (100 g) in Table 6.9. These values were obtained from the respirometry experiment carried out in the laboratory in section 6.2.2. While in the hypothetical site used in the study the mass of contaminants was 20,000 kg (diesel) in the 10,000 m^3 of soil. In order to arrive at the amount of $C0_2$ in the study here the initial value of CO_2 obtained in the laboratory was extrapolated. Therefore, if the treatment with soil plus diesel plus 20% BSG generated 2.97 g of CO_2 when 100 g of diesel was added then CO_2 that would be generated if 20,000 kg (20,000,000 g) of contaminants were remediated can be calculated to be 594 kg (2.97 * 20,000,000 /100). In order to calculate the CO_2e the value of 594 are converted into metric tonnes and the resultant value is multiplied by 1 which is the standard of Global warming potential (table 6.10). The calculation is as follows:

1 metric tonnes = 1000 kg

594 kg CO_2 = 0.594 metric tonnes CO_2

0.594 metric tonnes CO_2 * 1 = 0.594 metric tonnes CO_2e.

However, in rationalising the values of CO_2 in the study with treatment with BSG and treatment without BSG it was evident in the laboratory study that the addition of BSG emitted more CO_2 than when the soil has no nutrient amendment (Table 6.9). This will lead to a different time frame for the remediation target to be met. Since the goal of the research is to achieve the same remediation target of more than 90% of the diesel to be removed from the soil the bioremediation processes without the addition of BSG would achieve the remediation objective but later than the treatment with BSG (results of Chapter 4). But the $C0_2$ that would be emitted will be slower as indicated in the respirometry results and this value is 1.79 g for 129 days.

The value of CO_2 from bioremediation without BSG if 20,000 kg (20,000,000 g) of contaminants were remediated can be calculated to be 358 kg (1.79 * 20,000,000

/100). In order to calculate the CO_2e the value of 358 kg are converted into metric tonnes and the resultant value is multiplied by 1 which is the standard of global warming potential (table 6.10). The calculation is as follows:

1 metric tonnes = 1000 kg

358 kg CO_2 = 0.358 metric tonnes CO_2

0.358 metric tonnes CO_2 * 1 = 0.358 metric tonnes CO_2e.

Other emissions resulting from bioremediation include activities that result in CO_2, CH_4 and N_2O arising from the transportation of BSG from Hartlepool to Sunderland and turning of the soil during bioremediation. Table 6.12 and 6.13 below shows the *calculation of CO_2 CH_4 and N_2O resulting from transporting BSG from Hartlepool to Sunderland.*

Pollutants	Factors gCOe per vehicle KM	Kilometre travel	Emissions gCOe
CO_2 at 0% load	671.5	6,035	4,052,502.5
CO_2 at 100% load	863.4	6,035	5,210,619
Total		**12,070**	9,263121.5

Table 6.12: Loading vehicle factors for 0% and 100% which were applied to the kilometre travel to derive the emissions gCO_2 for BSG as a result of the vehicle movement from Hartlepool to Sunderland.

Pollutants	Factors gCOe per vehicle KM	Kilometre travel	Emissions gCOe
CH_4 at 0% and 100% load	0.36	12,070	4,345.2
N_2O at 0% and 100% load	7.75	12,070	93,542.5

Table 6.13: Loading vehicle factors for CH_4 and N_2O at 0% and 100% which were applied to the kilometre travel to derive the emissions gCO_2 for BSG as a result of the vehicle movement from Hartlepool to Sunderland.

Thus, the distance from Sunderland to Hartlepool has been determined to be 30 miles or 48.28 km (table 6.10). Since there was 2000 tonnes of BSG to be delivered using 16 tonne load lorries. It was estimated that it would take 125 trips to deliver the 2000 tonnes BSG. The total distance covered given the 125 trips would be 12,070 km.

However, there are different emission factors for running a vehicle empty and for full loads; as such 671.5 g was the emission factor for running a vehicle empty and 863.4g for running a full loaded vehicle (Table 6.10). Applying the different emission factors of running a vehicle empty and full load would amount to 4,052,502.50 gCO_{2e} and 5,210,619 gCO_{2e} for empty and full loading respectively. Therefore, the total CO_2 to transport the BSG from Hartlepool to Sunderland would be 9,263,121.50 gCO_2e (9,263.12 $kgCO_{2e}$). Similarly, the values of emissions for CH_4 and N_2O are calculated by multiplying their respective emission factors to the total distance covered to deliver the BSG from Hartlepool to Sunderland. From Table 6.13 the value for CH_4 is 4,345.20 g (4.345 kg) and N_2O 93,542.50 g (93.543 kg). As CO_2e are usually measured in metric tonnes the derived value is divided by 1000 and the resultant value is multiplied by 21 and 310 for CH_4 and N_2O respectively, which are the standard of global warming potential (table 6.10The calculation is as follows:

For CH_4

1 metric tonnes = 1000 kg

4.345 kg CO_2 = 0.004345 metric tonnes CO_2

0.004345 metric tonnes * 21 = 0.091 tonnes CO_{2e}

For N_2O

1 metric tonnes = 1000 kg

93.543 kg CO_{2e} = 0.093543 metric tonnes CO_{2e}

0.093543 metric tonnes * 310 = 28.998 tonnes CO_{2e}

In addition, CO_2 emissions with bioremediation were also associated with the use of 14 tonne excavator machinery, which has been estimated to consume diesel fuel of 910 litres for bioremediation with BSG and 1,330 litres for bioremediation without BSG.

Pollutants	Factors $kgCO_2e$	Amount diesel used/litres	Emissions $kgCO_2e$
Kg/CO_2e/Litre (bioremediation with BSG)	2.66	910	2,420.60
$KgCO_2e$/Litre (bioremediation without BSG)	2.66	1330	3,537.80

Table 6.14: Calculation of CO_2e derived by multiplying emission factors by the amount of diesel consumed for bioremediation with BSG and bioremediation without BSG as a result of the use of excavator machine.

Applying the emission factor for a heavy tractor of 2.66 kg (table 6.10) to the amount of red diesel consumed by the tractor during turning and tilling would amount to 2,420.60 $kgCO_2$ and 3,537.80 $kgCO_2e$ for bioremediation with BSG and bioremediation without BSG respectively. To derive the total CO_2e from bioremediation with BSG would be the summation of the CO_2 from combustion of fossil fuel by freight transportation of the BSG and CO_2 from turning the soil during bioremediation, which amounts to 11,683.72 kg CO_2e (2,420.60 kg + 9,263.12 kg). As CO_2e are usually measured in metric tonnes the derived value are divided by 1000. The calculation is as follows:

For bioremediation

1 metric tonnes = 1000 kg

11,683.72 kg CO_{2e} = 11.684 metric tonnes CO_2e

For bioremediation without BSG

1 metric tonnes = 1000 kg

3,537.80 kg CO_2 = 3.5378 metric tonnes CO_2e.

Pollutants	Factors g/ km	Vehicle Kilometre travel	Total Emissions g
Nitrox oxide (NOx)	3.870	12,070	46,710.9
Particulate Matter (PM_{10})	0.072	12,070	869.04
Particulate Matter ($PM_{2.5}$)	0.068	12,070	820.76
Carbon Monoxide (CO)	0.786	12,070	9,487.02
Volatile organic compound (VOC)	0.139	12,070	1,677.73
Ammonia (NH_3)	0.003	12,070	36.21
Sulphur Dioxide (SO_2)	0.003	12,070	36.21

Table 6.15: showing calculation of NOx, PM_{10}, $PM_{2.5}$, CO, VOC, CO, NH_3 and SO_2 emitted by vehicle movements through the delivery of brewery spent grain from Hartlepool to Sunderland.

Emissions from NOx, PM_{10}, $PM_{2.5}$, CO, VOC, NH_3, and SO_x which are recorded in g per km, emissions values in table 6.15 are derived by multiplying their activities data which is the distance covered for transporting BSG with their respective emission factors. The total distance travelled has been determined to be 12,070 km given the 125 trips and the emission factors can be found in Table 6.10. The value for NOx was calculated to be 46,710.9 g, PM_{10} 869.04 g for $PM_{2.5}$ estimated to be 820.76 g CO 9,487.02 g, VOC 1,677.73 g, NH_3 36.21 g, and SOx 36.21 g.

6.4.3. Natural attenuation processes (CO_2)

Few studies have evaluated the mass of CO_2 released during natural attenuation. However, over the estimated five year period for breakdown to occur it is likely that all of the hydrocarbon present will be broke down and released as CO_2.

However, the laboratory study using BSG showed that CO_2 generated when 20% BSG was added to the soil was 2.97 g at the end of 129 days and CO_2 generated without the addition of BSG was 1.79 g (table 6.9) at day 129. Natural attenuation

occurs without human intervention and CO_2 release will be slower than when the condition of the soil is modified such as with the addition of BSG. It was evident in the laboratory study that the addition of BSG emitted more CO_2 than when the soil has no nutrient amendment. Therefore, CO_2 from natural attenuation will be equated to that found in the laboratory study when no BSG was added because given the five years duration the same amount of CO_2 would be emitted but in a slower manner and the amount generated was expected to be equivalent to 1.79 g over a 5 year period.

In addition, the 5 year baseline adopted to achieve natural attenuation in the study could be regarded as a good situation for defining and assessing the baseline scenario because the data was obtained from a remediation company performance report, project manager and vendor (Sirius, 2010). Since the contaminant concentration was 20,000 kg the CO_2 that would evolve from the natural attenuation is expected to be equivalent to the amount generated when bioremediation without BSG was an alternative. This is because the process follows a similar pattern, as microbial activities result in the degradation of the organic compounds.

The results of the respirometry study have determined the value of CO_2 without the addition of BSG to be 1.79 g and the expected remediation objective was to be achieved in 9 months. Also, the same amount of CO_2 would be emitted if natural attenuation was adopted and the remediation objective was expected to be achieved in 5 years given the 20,000 kg of contaminants the total CO_2 would be 358 kg (1.79 * 20,000,000/100). In order to calculate the CO_2e the value of 358 kg are converted into metric tonnes and the resultant value is multiplied by 1 which is the standard of global warming potential (table 6.10). The calculation is as follows:

1 metric tonnes = 1000 kg

358 kg CO_2 = 0.358 metric tonnes CO_2

0.358 metric tonnes CO_2 * 1 = 0.358 metric tonnes CO_2e.

6.4.4. Landfill disposal option (CO_2)

Landfills have the potential for a range of negative impacts on the environment and human health, including a major cause of pollution to groundwater and surface water

across Europe (Defra, 2005). The presence of wastes in landfills will result in the decay of the organic matter and generation of CH_4 and CO_2. Landfills have been identified as significant anthropogenic sources of atmospheric CH_4 and are believed to account for 6-18% of the total CH_4 emissions in the world (Houde *et a*l. 1997). When oxygen is used up bacteria begin to produce CH_4. The more oxygen is present in a landfill, the longer aerobic bacteria can decompose waste (URL8). But if the waste is highly compacted (without oxygen), CH_4 production will begin earlier as the aerobic bacteria are replaced by methane-producing anaerobic bacteria (URL8) and CH_4 is generated in the landfill as the waste decomposes.

CH_4 lifetime in the atmosphere is much shorter than CO_2 but CH_4 is more efficient at trapping radiation than CO_2 (EPA, 2010b). The comparative impact of CH_4 on climate change is over 20 times that of CO_2 over a 100 year period (EPA, 2010b). However, CH_4 produced at landfills need not be released to the atmosphere but can be used as landfill gas. CH_4 and CO_2 landfill gas can be recovered and used directly in local electrical generators or as a feedstock for producing natural gas (EPA, 2010b). But in the calculation reported here breakdown of wastes in landfill will only be assessed as CO_2 production. However, wastes deposited in landfill are usually converted to both CO_2 and CH_4 and will eventually be expected to disperse to the environment as CO_2 and CH_4.

The emission of CO_2 from landfill in the study reported here would be calculated from the degradation of the organic compound. It was assumed in the study that 20,000 kg of contaminant was deposited in the landfill. The 20,000 kg of contaminant would be used as a proxy to evaluate the amount of CO_2 that would be evolved when the contaminated soil was landfilled. Although CO_2 from biodegradable waste does not pose a risk to the environment however if it emanates from hydrocarbons it would contribute to global warming and should be estimated when reporting emissions (IPCC, 2006).

Therefore, the 20,000 kg of contaminants depicted in the conceptual site model would be assumed to have been dispersed to the environment when landfill disposal was a possible option. Because depositing the contaminated soil to landfill will require a longer time for the waste to be degraded in the landfill the value of CO_2 adopted for natural attenuation and bioremediation without BSG will be used as the

same amount of contaminants that would have been emitted. This value is necessary because soil contaminated with diesel fuel is regarded as biodegradable waste and falls under the classification of hazardous waste (Defra, 2005) and in landfill deposited wastes are allowed to degrade over time and are converted to CO_2 and CH_4.

In the study here the value of CO_2e during bioremediation without BSG was previously estimated to be 358 kg. In order to calculate the CO_2e the value of 358 kg are converted into metric tonnes and the resultant value is multiplied by 1 which is the standard of global warming potential (table 6.10). The calculation is as follows:

1 metric tonnes = 1000 kg

358 kg CO_2 = 0.358 metric tonnes CO_2

0.358 metric tonnes CO_2 * 1 = 0.358 metric tonnes CO_2e.

There are also other CO_2 emissions associated with landfill disposal as a result of the movement of vehicles for the disposal and re-fill of the contaminated soil. The emissions are calculated by multiplying the distance travelled by the vehicle and the emission factors related to the journey. Table 6.16 and 6.17 show the calculations of CO_2 for disposing and re-filling the earth from Sunderland to Hartlepool.

Pollutants	Factors gCO_2e per vehicle KM	Kilometre travel	Emissions gCO_2e
CO_2 at 0% load	671.5	60,350	40,525,025
CO_2 at 100% load	863.4	60,350	52,106,190
Total		120,700	**92,631,215**

Table 6.16: Calculation of gCO_2e *emissions derived by multiplying emission loading factors of* CO_2 *to kilometres travelled during vehicle movement including empty and full load for disposal of the soil from Sunderland to Hartlepool.*

Pollutants	Factors gCOe per vehicle KM	Kilometre travel	Emissions gCOe
CO_2 at 0% load	671.5	60,350	40,525,025
CO_2 at 100% load	863.4	60,350	52,106,190
Total		120,700	**92,631,215**

Table 6.17: Calculation of gCO_2^e *emissions derived by multiplying emission loading factors of* CO_2 *to kilometre travel during vehicle movement including empty and full load for re-filling the site with new topsoil from Hartlepool to Sunderland*

The conversion factor for running empty Heavy Goods Vehicles (HGVs) and a full load were previously calculated as 671.5 and 863.4 respectively (table 6.10). It has previously been estimated in chapter 5 that it would take 384 tonnes of soil to be

disposed of daily using 16 lorry loads. The 20,000 tonnes of soil would require 1,250 trips to cover the entire journey and the distance from Sunderland to Hartlepool was previously estimated to be 48.28 km. In order to complete a journey the lorry is expected to run a full load and an empty load to dispose of the soil. Therefore, it will require 120,700 km to dispose of the 20,000 tonnes taking into consideration running the vehicle empty and full (48.28 X2 = 96.56 X 1250).

Since CO_2 are calculated based on the consumption of fuel used and the total distance covered for the entire journey. Table 6.16 and 6.17 shows the total distance travelled and the emission factors taking into consideration the consumption of fuel used when the vehicle is run empty and full and applying the related emission factors would amount to CO_2 of 185,262,430 g for disposing of the soil and for re-filling the site. In order to calculate the CO_2e the value of both disposing and re-filling are added together (92,631,215 g * 2 = 185,262,430 g) and converted into metric tonnes and multiplied by 1 which the global warming potential. The calculation is as follows:

1 metric tonnes = 1000 kg

185,262.43 kg CO_2e = 185.262 metric tonnes

185.262 metric tonnes * 1 = 185.262 metric tonnes CO_2e

Pollutants	Factors gCO_2e per vehicle KM	Kilometre travel	Emissions gCO_2e
CH_4 at 0% and 100% load	0.36	120,700	43,452
N_2O at 0% and 100% load	7.75	120,700	935,425

Table 6.18: Calculations of CH_4 and N_2O emissions by multiplying kilometre travel by emission loading factors including full and empty loading during disposing of the soil to landfill from Sunderland to Hartlepool.

Pollutants	Factors gCO_2e per vehicle KM	Kilometre travel	Emissions gCO_2e
CH_4 at 0% and 100% load	0.36	120,700	43,452
N_2O at 0% and 100% load	7.75	120,700	935,425

Table 6.19: Calculations of CH_4 and N_2O emissions by multiplying kilometre travel by emission loading factors including full and empty loading during re-filling of the soil from Hartlepool to Sunderland.

In addition, there were other emissions of CH_4 and N_2O that are calculated from the total kilometres travelled for disposing of and re-filling the soil. Table 6.18 and 6.19 shows the total values of CH_4 and N_2O calculated in gCO_2e per km. The total distance covered for disposing of the soil has previously been estimated to be 120,700 km including empty and full loads and the emissions factors of CH_4 and N_2O are the same values (table 6.10).

Applying the related emission factors would amount to 86,904g and 1,870850g for disposing and for re-filling the site for CH_4 and N_2O respectively. These include the net values from Table 6.18 and 6.19 for CH_4 (43,452 +43,452 = 86,904 g) and N_2O (935,425 + 935,425 = 1,870,850 g). As CO_2e are usually measured in metric tonnes

the derived value is divided by 1000 and the resultant value is multiplied by 21 and 310 for CH_4 and N_2O respectively, which are the standard of global warming potential (table 6.10).

The calculation is as follows:

For CH_4

1 metric tonnes = 1000 kg

86.904 kg CO_{2e} = 0.087 metric tonnes

0.087 metric tonnes * 21 = 1.827metric tonnes CO_{2e}

For N_2O

1 metric tonnes = 1000 kg

1,870.85 kg CO_{2e} = 1.871 metric tonnes CO_2e

1.871 metric tonnes * 310 = 579.964 metric tonnes CO_2e.

Other conventional emissions associated with vehicles movement were NOx, PM_{10}, $PM_{2.5}$, CO, VOC, NH_3 and SO_2. Vehicle movements for disposing of soil to landfill and re-fill of the site were already estimated to be 241,400 km which was a combination of a distance of 120,700 km for disposal of the soil and 120,700 km for re-filling of the site (table 6.18 and 6.19.).

Pollutants	Factors g/km	Vehicle Kilometre travel	Emissions g
Nitrox oxide (NOx)	3.870	241,400	934,218
Particulate Matter (PM_{10})	0.072	241,400	17,380.8
Particulate Matter ($PM_{2.5}$)	0.068	241,400	16,415.20
Carbon Monoxide (CO)	0.786	241,400	189,740.40
Volatile organic compound (VOC)	0.139	241,400	33,554.60
Ammonia (NH_3)	0.003	241,400	724.20
Sulphur Dioxide (SO_2)	0.003	241,400	724.20

Table 6.20: Calculation of NOx, PM_{10}, $PM_{2.5}$, CO, VOC, CO, NH_3 and SO_2 emitted by vehicle movement through disposing and re-filling of the soil from Hartlepool to Sunderland and values are derived by multiplying the kilometre travel by various emission factors.

Applying the various emissions factors to the distance travelled would result in the total emissions for a particular pollutant. Table 6.20 showed the total emissions for the different pollutants calculated in g per km for NOx is 934,218 g, for PM_{10} would be 17,380.8 g, $PM_{2.5}$ 16,415.20 g, CO 189,740.40 g, VOC 33,554.60 g, NH_3 724.20 g and SO_2 724.20 g.

6.4.5. Soil vapour extraction (CO_2)

SVE involves the contaminants being captured by a ventilation system, pre-heated, and thoroughly mixed and combusted at a high temperature. The contaminants are converted to innocuous end products in the form of CO_2 and water. Sufficient heating of a volatile organic compound in the presence of oxygen will convert the VOCs to a harmless end product (AICE, 1993 and U.S EPA, 2006.).

Using SVE treatment facility such as thermal treatment technologies could destroy hydrocarbons in a vapour stream at an elevated temperature by oxidizing the hydrocarbons to CO_2 and water (U.S EPA, 2006). Catalytic oxidation is regarded as the most common thermal treatment off-gas technology; the system uses a catalyst together with heat to oxidize contaminants in a vapour stream (U.S. EPA, 2004). The

study reported here will use this system to oxidize the 20,000 kg of contaminants and the addition of catalyst will accelerate the rate of oxidation by adsorbing oxygen and the contaminants on the catalyst surface, where they react to form CO_2 and H_2O. The chemical reaction for hydrocarbon oxidation is defined by U.S. EPA, (2006) as:

$H_2C + O_2$ + heat >>>>> $CO_2 + H_2O$ + heat

Where

H_2C = hydrocarbon

O_2 = Atmospheric oxygen

CO_2 = Carbon dioxide

H_2O = water

And chemical reaction for diesel

$C_{16}H_{34} + 33O_2$ >>>>>> $16CO_2 + 17H_2O$

Therefore, the thermal oxidation treatment would result in more than 95% destruction of the contaminants and they would eventually disperse to the atmosphere as CO_2 and water. The by-product of this destruction is referred to as the product of complete combustion, if they are combusted with sufficient oxygen and these compounds are innocuous and can be discharged directly to the atmosphere (U.S.EPA, 2006). Using this technology in the study would assume that all the CO_2 will be captured and brought to the surface facility as gases.

Furthermore, it is necessary to treat all SVE off-gas for almost all facilities because the vapour gases or the volatile organic compounds cannot be dispersed to the environment due to their harmful effects (U.S. EPA, 2006). Treating the gases before discharge to the atmosphere is the most expensive portion of the systems operating costs due it its high cost of energy to heat the liberated gases. The evaluation of a case study in chapter 5 of a typical off-gas treatment using SVE found that 130 pounds (59.1 kg) contamination extraction rate was achieved per day and during the year of operation, the oxidizer destroyed 16,000 pounds (7272.7 kg) of gasoline vapours. A draft by U.S. EPA by the office of Superfund remediation and technology

innovation estimated electricity consumption and CO_2 emission for typical SVE system in U.S. In the analysis the annual estimated electricity use for 3 years was 232,148 kWh (U.S. EPA, 2008a) and this estimate has been used to project CO_2 by the U.S. EPA from 2008 to 2030.

However, a similar evaluation carried out in 2010 using best management practices (BMPs) and the sampling of electricity consumed by SVE components for three years excluding system design and construction was estimated to be 232,800 kWh (U.S. EPA, 2010a). This could emit 184 tons of CO_2 based on the average U.S fuel mix, which is equivalent to the electricity used by nearly 22 homes a year (U.S. EPA, 2010a).

In the study here using the same SVE components (table 6.10) such as thermal oxidation where the treatment facility will oxidize the diesel pollutants and convert it into CO_2 and water. The harmless products would be discharged to the atmosphere and it would be monitored by the use of a continuous detection systems (FID) used by AAA construction (URL7) where any incomplete combustion will be checked and treated accordingly and in compliance with the limit of the laws.

Since 20,000 kg of contaminants will be destroyed in a 3 year period for the remediation objective to be met, it would require the same amount of energy of 232,800 kWh specified above to destroy all the contaminants. But the use of this facility will generate CO_2, due to electricity consumption and CO_2 emitted/kWh generated is 1.37 Ibs or 0.6214 kg (table 6.10). Convertibly the SVE treatment during the 3 years of operation will generate a total CO_2 of 144,661.92 kg (232,800 * 0.6214). In order to calculate the CO_2e the value of 144,661.92 kg are converted into metric tonnes and the resultant value is multiplied by 1 which is the standard of global warming potential (table 6.10). The calculation is as follows:

1 metric tonnes = 1000 kg

144,661.92 kg CO_2 = 144.662 metric tonnes CO_2

144.662 metric tonnes CO_2 * 1 = 144.662 T CO_2e.

6.4.6. Thermal desorption

In the in-situ thermal desorption facility, vaporization is the main mechanism used to

enhance recoveries of volatile organic compounds. The in situ thermal desorption method had been designed and implemented and consistently produced more than 95% mass removal in clay soil (Baker *et al.* 2008). The technique in the present study involves the simultaneous application of heat by thermal conduction and SVE to remove the diesel from the soil. The processes involve using recovery wells to capture both liquids and gases and transport them to the subsurface facility for treatment. Thus, various residuals are usually generated from the thermal process. Some of these are hazardous others are non-hazardous, but diesel fuels are certainly hazardous and would be condensed to a liquid form or treated as off-gas.

In the case study reviewed in chapter 5 the energy consumption during in situ thermal desorption when 11,100 m^3 of soil volume for 6 months was treated was 342 kWh/m^3. This corresponds to 3,796,200 kWh of electricity use for 11,100 m^3 when all the components that used electricity were included. According to the review conducted by the U.S. EPA, (2008b) where all the components that use electricity or energy were reviewed and analysed at Superfund clean-up sites using five remediation technologies including thermal desorption to treat contaminated material such as hydrocarbon, it was found that electricity usage accounts for 89% of total energy cost. The amount of electricity use accounted for 95% of total CO_2 emissions and fuel accounted for 5%.

In the study here if the same technology that was used to remediate the 11,100 m^3 in the Reerslev site in Denmark, was used, the remediation target could be achieved in 6 months. Converted to the actual soil volume of 10,000 m^3 in the study here in order to achieve up to 95% clean-up level for 6 months using the same in situ thermal desorption facility, would require predicted energy of 3,420,000 kWh of (342 kWh/ m^3 * 10,000). The contaminant would be captured and destroyed in an afterburner which is a surface facility to treat off-gas materials. The amount of contaminants to be treated was previously estimated to be 20,000 kg of organic compounds. But CO_2 emitted per kWh of electricity was estimated to be 1.37 lbs (0.6214 kg) as indicated in table 6.10. Then the thermal desorption facility in the study is expected to operate for six months and will generate a total CO_2 of 2,125,188 kg (3,420,000 x 0.6214 kg). In order to calculate the CO_2e the value of 2,125,188 kg are converted into metric tonnes and the resultant value is multiplied by 1 which is the standard of global warming potential (table 6.10). The calculation

is as follows:

1 metric tonnes = 1000 kg

2,125,188 kg CO_2 = 2,125.19 metric tonnes CO_2

2,125.19 metric tonnes CO_2 * 1 = 2,125.19 T CO_2e.

6.5. Impacts of remediation activities on water and land

Pollution incidents are most likely to lead to water or land damage where the incident results in serious contamination of soil on the surrounding land. In addition, the mitigation action put in place should not contribute additional damage to the land or surrounding water. The majority of times environmental damage may be in the form of new pollution, as a result of chemical use, during the period of remediation or from transferring the contaminants to a different location where it may affect surface water or underground water. In the study here the hydrocarbon contamination does not affect underground water or surface water as shown in the hypothetical site. However, the adoption of any remediation method could result in contamination of underground water or surface water.

Over the years, many methods for dealing with contaminated soil have been developed. Increasingly regulators and remediation practitioners are now considering the assessment of the risk that may follow different remediation methods (EA, 2004). Therefore, it is always important to consider not only the risk posed by an existing situation, but also the emerging risk that might arise from the action of a given remediation technique (EA, 2004). Table 6.21 and 6.22 describes the potential impacts of the five remediation options considered in the study in relation to water and land. The effect of each of the remediation methods are described in the next

Remediation methods	Potential impacts on water course
Bioremediation With and Without BSG	Bioremediation processes usually lead to leaching, which may affect any possible water course. In the study bioremediation with or without brewery spent grain may result in pollution of nearby rivers (surface water) and could also contaminate underground water.
Natural attenuation	The choice of allowing the soil to remediate itself using indigenous microorganisms could pose a potential risk both now and in the future to surrounding watercourses. Although there is no current migration of contaminants to underground water or surface water the possible risk is high should the hydrocarbon contamination be left on site. This is because if the unsaturated zone is not remediated contamination will migrate downward and contaminate groundwater.
Landfill disposal	The landfill method means the contaminated soils are excavated and disposed in landfill and these eliminate risk of underground and surface water contamination on the contaminated site. However, landfill disposal location (facility or sites) are usually associated with leachate resulting from decomposed organic materials.
Soil vapour extraction (In-situ)	During soil vapour extraction contaminants in form of liquids and gases can diffuse slowly from less permeable soils and interact with soil gas and groundwater (U.S EPA, 2006). Also there is the potential risk of the contaminants leaching to underground water during the extraction process.(U.S AFERP, 2001).
Thermal desorption(in-situ)	During thermal desorption contaminants could migrate to underground water during extraction processes due to soil gas pressure and fluid elevation within and outside the treatment zone (Johnson et al., 2009).

Table 6.21: The five remediation options and their possible impacts on underground water and surface water for five different remediation options

Remediation methods	Potential impacts on land and facility
Bioremediation With and Without BSG	The process could impact surrounding buildings as a result of leachate from contaminated soil mixing with BSG. There may be potential risk of leachate to underground facility or water because the tilling and turning of the soil are carried out above surface. Although any leachate migrating out of the treatment site are collected and treated see risk assessment table in section 5.1.3 and appendix IV.
Natural attenuation	The process could impact on any facility or land due to the possibility of leaching. However, the technique is non- intrusive with limited human interference. But as the technique relies on the indigenous bacteria to degrade the soil the process will be slow and uncontrollable.
Landfill disposal	The process does not constitutes any negative impacts on the site as the contaminated soil are excavated and disposed of and new soil are used to fill the earth.
Soil vapour extraction (In-situ)	The process could be vulnerable to a high risk to the nearby structure and underground utilities from the high level of the volatile compounds (diesel). There could be also explosive risk in nearby structure and underground utilities from high level of volatile organic compounds and risk from direct contact with soil vapour such as the exposure to utility installation worker to contaminated soil. (U.S. AFERP, 2001).
Thermal desorption (In-situ)	There is typical concern about geotechnical stability and damage to foundations, building or underground utilities. However, control measures are usually in place as cases are dealt with relatively easily on a site-specific basis (Johnson, et al., 2009).

Table 6.22: The five different remediation options and their possible impacts on land.

6.5.1. Bioremediation with and without BSG effect on land and water pollution

The adoption of a bioremediation technique could result in leachate percolating through the soil and possibly entering into the nearby water course as a result of uncontrolled discharge of suspended sediment as indicated in the hypothetical site. The leaching may contain the organic waste from the BSG and the hydrocarbons from the soil and water running off the compacted soil may contribute to diffuse

pollution as indicated in table 6.21. This assessment is in accordance with (SuRF, 2010) guidelines which indicate that remediation impacts on water may result from the release of contaminants such as nutrients and dissolved organic carbon which will impact on the quality of water.

In addition, the benefits of bioremediation include safe operations, facilitated materials balance and process controllability (U.S. EPA, 2008b). In the study here the processes were carried out above ground as tilling and turning of the soil are carried out on the surface but the potential of leaching to occur from the mixture of BSG and contaminated soil may affect under or above ground water as shown in the case study site and indicated in table 6.22. However, control measures could be put in place to collect leachate from the soil, in the form of trenches excavated around the treatment location to collect any escaping leachate, which is then sent for treatment. The risk assessment model in section 5.1.3 addresses the potential risk of leaching from bioremediation in the study.

6.5.2. Natural attenuation effect on land and water pollution

Natural attenuation technology is referred to as intrinsic bioremediation (Borden *et al*. 1995) because the process relies on the natural assimilative capacities of the ground to act on the contaminants (Simarro *et al.* 2013).The technique also relies on indigenous microorganisms to degrade the contaminants (Suni and Romantschuk, 2004). The process seems slow and uncontrollable as there is limited human interferences. There would be no damage to the land or aquifer as a result of use of the technique as it does not involve the use of heavy machinery or chemicals that may lead to additional burdens on the soil.

However, climate scenarios in the U.K. such as wetter winters, hotter and drier summers, rising air temperature, increased storminess and heavier rainfall (Hulme *et al.* 2002) could have impacts on technique such as natural attenuation. Thus, severe weather conditions may have damaging effects on soils with potential impacts for any ground contamination especially shallow untreated contaminated sites as depicted in this study in that during winter there may be a seasonal rise in groundwater levels which can bring clean groundwater in contact with the diesel contaminants. More so, higher intensity rainfall will challenge soil infiltration capacity and double the risk of soil erosion and spread of the diesel oil beyond the expected

area.

Therefore, the remediation method of natural attenuation in the study may result in water and land pollution although the hypothetical site shows that the contamination does not affect the saturated zone and the surface water course. However, since the natural attenuation will take a minimum of 5 years to achieve based on the remediation schedule in this study there is an increasing risk of contamination from eroded soil if the environmental conditions change over time for instance flooding could impact on surface water and contribute to pollution of site drainage water and the surrounding land.

6.5.3. Landfill disposal effect on land and water pollution

The adoption of landfill remediation may not lead to any water or land pollution within the site as the soil would be excavated and disposed in a landfill and clean soil would be used to replace it. The process involves the contaminants being transferred to a different location. The volume of contaminated soil at the site in Sunderland is 20,000 tonnes and a total of 16 vehicles will require 1,250 trips to dispose of the soil. In addition topsoil was used to fill the areas of the site where the contaminated soil has been removed. The dig and dump method does not have any impact on the water or surrounding buildings but may increase the risk to human health and environment through traffic accidents and exposure to dust (EA, 2004).

6.5.4. Soil vapour extraction (SVE) effect on land and water pollution

The remediation technique of SVE considered in the study here would remove the contaminants from the soil and destroy them using an above ground treatment process. The treated vapour stream would contain undesirable by-products but they are the products of complete combustion, CO_2 and H_2O and they can be released to the atmosphere because the compounds are innocuous (U.S. EPA, 2006) and may not have an effect on land and water Using SVE involves several mechanisms but recovery wells are used to capture both liquid and gases and transport them to the surface facility for treatment. During the engineering process of air injection there is the possibility of dangerous vapour migration into buildings or underground utilities but control processes are usually put in place to mitigate against such an occurrence. More so, vapour treatment facility is usually designed to meet air

discharge regulatory requirements however there could be a risk to groundwater based on the leaching of contaminants from soil during the extraction process.

6.5.5. Thermal desorption effect on land and water pollution

Thermal desorption considered in the study would produce secondary waste streams including solids, water condensate and oil condensate, each of which may require analysis to determine the best disposal/recycle options. During the thermal desorption process there may be the possibility of an explosive risk to nearby structures and underground utilities from high levels of volatile organics. This is because the technique would use a non-oxidizing process to vaporize the hydrocarbon through the application of heat and as a result of drilling the earth. It may impact the nearby structure with a risk of high level explosions due to the volatile nature of the contaminants. But these have not been a significant barrier to in situ thermal desorption techniques as the concerns regarding land and water pollution are addressed as part of the project implementation process (Johnson *et al*. 2009).

However, due to the high temperature used to treat the contaminants the treated soil would be subjected to weight loss as a result of pyrolysis of the petroleum products in the soil and even soil that does not have petroleum contaminants in the surrounding treatment zone may lose weight significantly as temperature rises. Thus, weight loss could be caused by not only moisture evaporation but also by decomposition of humus materials. In addition there may be some minerals such as carbonate salts found in the soil, which could be subjected to high temperatures by the soil vapour extraction technology (Lee *et al.* 1998). In addition, contaminants can diffuse slowly from less permeable soil and interact with soil gas and groundwater, which may occur during pyrolysis (U.S. EPA, 1996a). Thus, there is a potential of underground water or land pollution as the process generates waste streams such as spent carbon and condensed water, which may be disposed in landfill.

6.6. Discussion

During remediation of contaminated soils there are a range of environmental impacts associated with different remediation techniques. For example bioremediation has been associated with leachate from decomposing organic matter percolating into the

soil and possibly into nearby watercourses and the potential release of any micro-organisms into the wider environment (Komilis and Ham, 2006).

In addition, bioremediation techniques involve the use of heavy machinery such as excavator/tractors, petrol and diesel-engine motor vehicles. The use of these machines, poses a major threat to clean air as they emit a wide variety of pollutants including CO, NOx, VOCs and PM_{10}, which have an increasing impact on air quality (Defra, 2010).

In the context of soil remediation environmental burdens caused by the remediation itself includes impacts on resource consumption, energy usage, transportation emissions, and toxicity (Ellis and Hardley, 2009). Hence in the present study it is important to establish the magnitude of emissions from the range of remediation techniques considered in the study and evaluate which technique is more sustainable if applied under the same conditions. The discussions of the findings are presented below:

6.6.1. Process and activities emissions

There are two classifications of emissions under review in the study here - process and activities based emissions. The process based emissions were referred to in the study as emissions resulting from the diesel contaminants during remediation, which may be vaporised/volatised or used by micro-organisms and converted to CO_2 and water during remediation. It is emissions that result from the contaminant (diesel) directly during remediation. Whilst the activities emissions are as a result of the use of energy or electricity during the period of remediation these include the use of heavy goods vehicles or heavy machinery during remediation. The activities emissions were as a result of remediation activities and not from the diesel contaminants in the soil. These two classifications are discussed below:

6.6.2. Process emissions from bioremediation technique

Soil respiration is an influential component of the biosphere's carbon cycle as it makes up about three-quarters of total ecosystem respiration (Law *et al.* 2001). Soil respiration is a major aspect of soil-quality and an indicator of soil fertility (Staben *et al.* 1997). But the release of CO_2 from the soil has been in the front line of intense studies in recent years, due to its controversial aspect on global warming potentials

(Luo *et al*. 2001). Therefore, measuring soil CO_2 respiration is a medium to assess biological soil fertility. Bioremediation processes involve the degradation of organic compounds in the soil and may result in CO_2 being emitted (Walworth and Reynolds, 1995) especially when it is enhanced by the addition of organic wastes such as BSG and this may have an impact on the environment.

In a study conducted in the laboratory by Benyahia *et al.* (2005) using the method of measuring CO_2 based on the standard titrimetric determination of CO_2 trapped in a concentrated NaOH solution, the bioremediation process yielded a much greater respiration rate and resulted in a reduction of 75% of oil in the soil in 118 days.

In the study here the respirometry experiment was carried out to determine the volume of CO_2 evolved in the presence and absence of BSG. This information was then used to review the environmental costs associated with the process. It has been asserted by (Reddy and Adams, 2010) that measuring environmental impacts of remediation should include metrics such as CO_2 emission per unit of treated soil (tonne/kg), energy per unit treated mass (kWh/kg), air pollution (kg or tonnes), waste generation (kg/tonnes) and water consumptions (m^3 or gallons). In terms of the calculation of environmental costs there is a need to ascribe actual values to the various factors costed (Defra, 2008).

The results from the respirometry in the study indicate that addition of BSG results in higher microbial activity, as indicated by an increase in emissions of CO_2, which was higher than the treatment without BSG this might be expected as the microbes will break down the spent grain as well as the diesel contaminating the soil.

From the results in this study BSG will be beneficial in terms of its ability to enhance the bioremediation process but it could result in a greater environmental impact, in terms of the release of greenhouse gas emissions.

The adoption of the respirometry technique described here could be one means of ascribing a value to environmental impacts, in terms of the release of CO_2. The quantitative measurement of CO_2 can then be added to the social and economic costs to determine how sustainable a remediation option could be (SURF-2010). The value of process emissions from bioremediation with and without BSG has been estimated to be 0.594 TCO_2e. and 0.358 TCO_2e respectively (Table 6.11).

6.6.3. Process emissions from natural attenuation and landfill disposal

CH_4 and CO_2 are generated in landfill as waste decomposes. In the study here focus was only on CO_2 which was previously calculated as 0.358 TCO_2e (Table 6.11). But landfill gas can also generate economic benefits for the community as it reduces the need to use more polluting energy from coal and oil when landfill gas is produced. Whilst CO_2 from natural attenuation has been previously calculated from the anaerobic decomposition of the organic waste to be 0.358 TCO_2e (Table 6.11)

6.6.4. Process emissions from SVE and thermal desorption

In the past thermal, chemical and physical treatment methods have failed to eliminate the pollution problem because these techniques only transfer the pollution to a new phase such as air pollution. But with the use of a thermal facility such thermal oxidizer, which is equipped with a heat exchanger and the combustion gas is used to pre-heat the incoming contaminated gas (FRTR, 1999), means vaporization is the main mechanism used to enhance recoveries of volatile organic compounds. Thus, various residuals are usually generated from the process and in the study it would be hazardous because diesel fuels are certainly hazardous, which would be condensed to liquid and treated as off-gas. The in situ thermal desorption method had been designed and implemented and consistently produced more than 95% mass removal in clay soil (Baker *et al*. 2008).

Therefore, the thermal oxidation treatment would result in more than 95% destruction of the contaminants and they would eventually disperse to the atmosphere as CO_2 and water. The by-product of this destruction is referred to as the product of complete combustion, if they are combusted with sufficient oxygen and these compounds are innocuous and can be discharged directly to the atmosphere (U.S.EPA, 2006). Using this technology it is assumed that all the CO_2 will be captured and brought to the surface facility as vapour gases and uses recovery wells to capture both liquid and gases.

The treated vapour stream would contain undesirable by-products but they are the products of incomplete combustion (U.S.EPA, 2006). Thus, sufficient heating of the volatile organic compound in the off-gas facility in the presence of oxygen will convert the VOCs to a harmless end product (AICE, 1993 and Suthersan, 1999).

These can be released to the atmosphere as CO_2 and water subject to meeting the requirement of local air discharge regulations (U.S.EPA, 2006).

Thermal oxidation equipment is currently used for destroying contaminants in the exhaust gas from air strippers and SVE systems (FRTR, 1999). VOCs are thermally destroyed at temperatures ranging from 600 to 1000^0C using a solid catalyst. Initially, the contaminated air is directly pre-heated to reach a temperature necessary to initiate the catalytic oxidation of the VOCs. Thereafter, the pre-heated VOC-laden air is passed through a bed of solid catalysts where the VOCs are rapidly oxidized. The effluent is either discharged or pumped back as a fluid recycling and the VOCs in the gas stream are completely destroyed (FRTR, 1999).

However the thermal oxidation system could use a chamber system to combust the VOCs as described in NFESE, (1998) where contaminants are vaporized in the treatment chamber and swept away by the air stream and as they pass through a burn chamber they become part of the combustion process and the contaminants are destroyed. The VOCs actually serve as a form of supplement fuel in the burn chamber; helping to heat the circulating gas stream (URL7) and emissions from the chamber in the study here are regarded as part of the activities emission which are discussed in the next section.

More so, SVE in the study here would adopt a similar technology of catalytic or thermal oxidation to treat the air stream laden with volatile compounds (VOCs). Heat recovery equipment would be installed with a thermal oxidation system to pre-heat the VOC-laden air stream as described in (Suthersan, 1999). Pre-heating the incoming stream reduces the amount of fuel that is needed to maintain the combustion temperature as the VOCs would be used as supplementary fuel. Although low concentration VOCs streams may not have the oxidation energy required to maintain the combustion temperatures there is a need for other source of energy. In the case study site the 20,000 kg of diesel contaminants in the soil would be combusted and used as a supplemental fuel in addition to other sources of energy to provide energy for the SVE facility. Although, there may be an element of process emission (VOC's) associated with thermal desorption and SVE system. These emissions were considered as minimal in the study because the facility used to treat the captured gases or liquid as the VOC laden air was captured by a

ventilation system, pre-heated, thoroughly mixed and combusted at high temperature.

In the study emissions resulting from VOC's were not evaluated for SVE and thermal desorption rather they were considered as emissions resulting from the use of energy, which were grouped as part of the activities emissions. Thus, process emissions were recorded for bioremediation with and without BSG, natural attenuation and landfill disposal option. The next sections will discuss the emissions resulting from remediation activities such as transportation and the use of energy to arrive at the total emissions measured as CO_2e.

6.7 Activities emissions from transportation, use of energy and other remediation activities

Bioremediation with BSG and other remediation options in the study have emissions resulting from the use of heavy good vehicles and energy from electricity. For bioremediation with BSG and without BSG, emissions from the use of heavy goods vehicles and excavator machines for turning and tilling the soil has been estimated in terms of CO_2 (section 6.4.2). The addition of these emissions to other gases such as CH_4 and N_2O are converted into their Global Warming potential (GWP). These three gases represented as a single value allow different emissions from other remediation methods to be compared using their GWP. The results section of Table 6.11 has already converted the three gases into CO_2e for the different remediation options including bioremediation with BSG. Figure 6.3 below shows the comparison of the CO_2e of bioremediation with and without BSG to other remediation options considered in the study such as landfill, natural attenuation, SVE and thermal desorption.

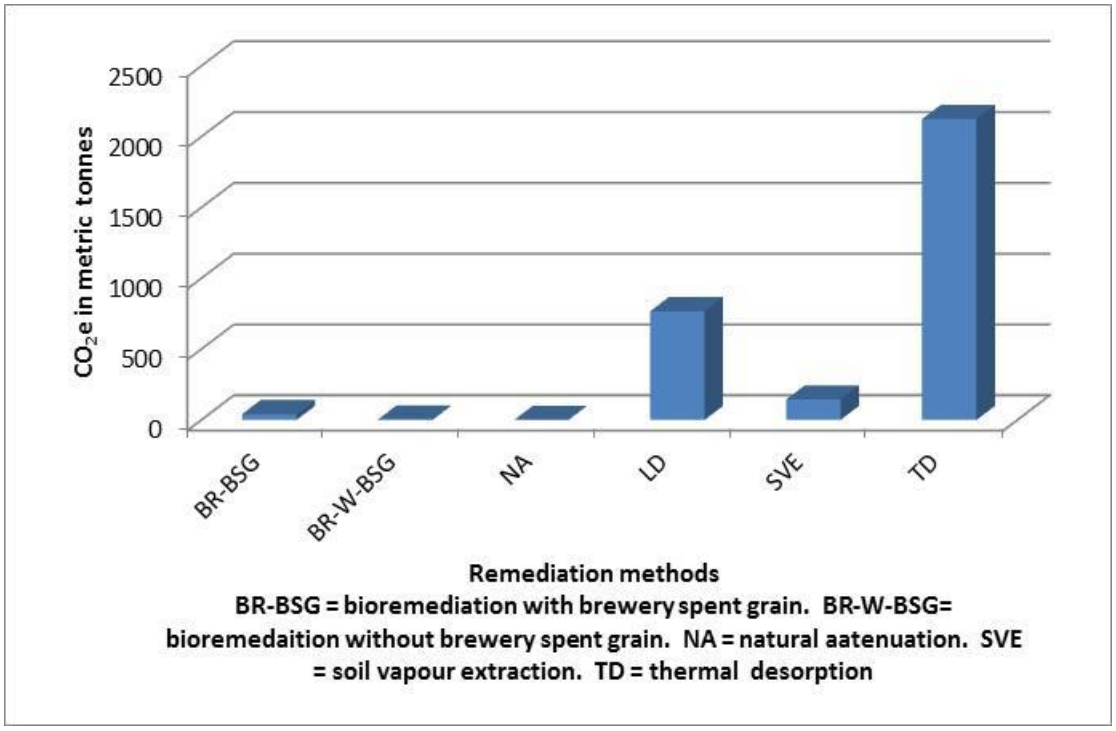

Fig.6.3: Summarises the pollutants value of emission from remediation and activities for the different remediation options consider in the study with estimation of their CO_2e.

From Fig. 6.3 bioremediation with BSG was slightly higher than bioremediation without BSG and natural attenuation because of the movement of heavy goods vehicles used to transport the BSG from Hartlepool to Sunderland. Landfill disposal and thermal desorption were much higher than bioremediation due to the high energy demand of the technology and their associated high CO_2 emissions.

However, the results of this study were not in agreement with the study of Baker *et al*.(2011) in which life cycle assessments were conducted at five sites where in situ thermal desorption was compared with SVE and landfill disposal and the values of CO_2, CH_4 and N_2O were converted as a single value (CO_2e) with their GWP. In that study thermal desorption in each instance had lower overall environmental impacts and costs. The reasons for the lower environmental impacts of thermal desorption in the Baker study was that it would require 240 km to dispose of the soil to landfill and SVE was expected to be completed in 100 years due to the difficulties of implementing the technology in that particular site, as compared to 3 years in the study reported here.

In the study here thermal desorption was compared to process based technology such as bioremediation and there are few or no studies that have made this analysis because of the difficulties of measuring the environmental footprint of bioremediation. In addition, the traditional methods of SVE and landfill presented in this study differ from the Baker study in that the distance covered from Hartlepool was estimated to be 48 km and SVE average completion time in the UK is 3 years (Defra, 2010). According to the results in this study thermal desorption, landfill disposal and SVE produced heavy impacts while bioremediation yielded lesser but different impacts. This makes bioremediation more comparable in terms of environmental cost and thermal desorption the least favourable environmental option.

Landfill disposal has higher CO_2 emissions compared to other remediation options in the study including bioremediation with/without BSG, natural attenuation and SVE. Thermal desorption in the results reported here has higher CO_2 compared to landfill disposal but landfill is associated with CH_4 the impact of which is greater on climate change, over 20 times that of CO_2 (EPA, 2010b). This may make landfill disposal less attractive when compared to bioremediation, natural attenuation and SVE. It also an indication that economic activities that could lead to higher greenhouse gas emissions today are likely to have consequences far into the future (Defra/DECC's, 2011).

6.8 Other conventional air pollutants

There are emissions, which have increasing impacts on urban air quality as a result of emission from motor vehicles. The effects of these emissions on air quality are almost entirely due to vehicle exhaust emissions (Atkin, Report 2007). These are major threats to clean air and are emitted by petrol and diesel-engine motor vehicles (Defra, 2010). In the study here these pollutants are only applicable to landfill and bioremediation as they involved the use of motor vehicle to transport contaminated soil and BSG. Table 6.11 provides summary values of these emissions emitted to the atmosphere during remediation activities, which include NOx, PM_{10}, $PM_{2.5}$, CO, NH_3 and SO_2. According to the EC (2013) humans can be adversely affected by exposure to air pollutants in ambient air. Due to their health effects the European Union has developed an extensive body of legislation that established health based standards

to limit the values emitted or concentrations in the atmosphere.

In the study here these emissions, which are associated with landfill and bioremediation methods were measured. Other remediation options such as thermal desorption and SVE, which emit similar forms of pollutants including CO and some oxides of substances like sulphur and nitrogen were not calculated as they are completely or partially eliminated during the remediation process (U.S. EPA, 2006). For example thermal desorption facility used by AAA Construction and Development (URL7) used a system of continuous detection air emissions to monitor pollutants such as CO and substances like sulphur and nitrogen coming from the afterburner facility to ensure compliance with the limit of the law and eliminate such hazardous substances. The facility used for thermal desorption is made to follow the ATEX Directives. ATEX is a conventional name for Directives 94/9/EC of the Europeans Union for the regulation of equipment intended for use in hazardous area (URL7). Whilst SVE equipment is subject to regulation of the ATEX Directives and the facility is expected to meet the emission standard for NH_3 and CO, because of the insignificant nature of the emissions from the engines use to power the SVE facility the calculations are not considered in the study here.

The landfill disposal option emitted more of these pollutants than bioremediation due to the distance covered to dispose of the contaminated soil, which was 241,400 km, whilst bioremediation covered a total distance of 12,070 km, however, these pollutants should be treated based on their individual merit because of the air quality standard set by European Commission on the concentration of these pollutants in the atmosphere. According to the EU Daughter Directive, (EU, 2013) the limit value of NO_2 is 200 µg/m^3 in 1 hour and 50 µg/m^3 in 24 hours for PM_{10}. The concentration value for $PM_{2.5}$ is 25µg /m^3 in 1 year and CO is set for 10 µg /m^3. The limit value for SO_2 is set for 350 µg /m^3 and there is no limit value for NH_3 because it is not regulated by the Directive.

In practice emissions from these pollutants are not considered during remedial selection but Defra, (2010) recommend that these should be calculated as part of sustainable remediation. Exceeding EU concentrations due to remediation activities seems practically impossible but remediation activities such as movement of vehicles could contribute immensely to the limit values. In the study using HGVs to

dispose of the contaminated soil to landfill was estimated to take 1,250 trips from Sunderland to Hartlepool. Hot exhaust and cold start emission for 1,250 trips using HGVs diesel engines should be considred, in terms of pollutants emitted from collection to disposal points. Whilst the bioremediation technique has been estimated to be 125 trips to deliver the BSG from Hartlepool to Sunderland although the number of trips is lower compared to landfill but the collection and disposal points need to be considered in respect of the EU limit value.

In the study here emission values for NOx, PM_{10}, $PM_{2.5}$, CO, NH_3 and SO_2 resulting from the movement of vehicles for transporting BSG are minimal compared to landfill. But in practice consideration should be given to the location and time of activities because exceeding these values are based on measurement per m^3 in a particular area and the averaging period depends on the pollutants under consideration. However, the values of these emissions in the study between bioremediation with BSG and landfill disposal option are comparable. Despite the uncertainty of the concentration of these emissions in various locations covered in this study the total sum of the impacts meant that with regard to overall evaluation of NOx, PM_{10}, $PM_{2.5}$, CO, NH_3 and SO_2, landfill seems to be environmentally the most undesirable option for removal of the diesel from the site, compared to bioremediation when vehicle miles covered by the two remediation methods are considered.

6.9 Water impacts

In the study here the six remediation options considered were in-situ and ex-situ such as landfill, which entail excavating and transporting the contaminated soil to designated landfill. Bioremediation with and without BSG and natural attenuation could significantly alter the ambient aquifer pH and nitrate concentration if there is migration to the surrounding water course or beyond the treatment zone in the case of flooding during bioremediation. For instance the mixture of BSG added to the diesel contaminated soil could migrate to the sub-surface water. In the study here the soil pH or the relative acidity of the soil was measured in the laboratory in chapter (4) and the microbe colonies were able to thrive in the soil. This is because a fairly narrow pH range encourages microbial colonies (U.S. DOE, 1996). This may not be the case in the hypothetical site model as the soil tested in the laboratory had diesel

concentrators of 5,000 mg/kg^{-1} soil and 10,000 mg/kg^{-1} soil for experiment (a) and (b) respectively. However, in the case study site the diesel concentration was previously estimated to be 20,000 kg in the 10,000 m^3 soil and this may pose a potential environmental hazard if there was leaching to the surrounding water course as hydrocarbons such as diesel may be expected to have a lower pH with properties of a weak acid (U.S. DOE, 1996).

There may be little or no impact of thermal desorption on ground water because heating the soil generally reduces the solubility of the diesel preventing migration to ground water and the contaminants would be captured as vapour gasses and brought to the surface for further treatment. The same can be said of SVE where the contaminants are captured and treated in an off-gas treatment facility without migrating to underground water.

The landfill disposal option simply moves the contaminants to a different location without having any impacts on the surrounding water course. However, during digging or excavation of the soil with heavy machinery there is the possibility that the soil could be damaged and could lead to a source of water pollution. But this is normally dealt with as part of implementation process.

When considering in-situ technology Defra, (2010) recommend that potential contaminants that can significantly alter the pH of the surrounding water course or beyond the treatment zone should be evaluated. The impacts on water are not considered in the study reported here as there was not time to investigate this aspect in detail, future work could include an assessment of the constituents of leachate generated by the bioremediation process. While the diagnosis of the nature of the impact of water is an essential prerequisite for successful remediation of contaminated land, in many cases some type of evaluation will be required prior to the remediation work. As there are many different types of impacts on water, so there are many different forms of solution.

6.10 Land impacts

One of the aims of this research is to achieve the clean-up of soil contaminated with diesel oil and the remediation options considered have been adjudged to be capable of achieving more than 90 per cent clean-up level. Complete removal of the diesel

from the soil with a particular remediation technique may alter the soil organic matter and lead to biological perturbation. In the study here quantitative values will not be assigned to any impacts resulting from land as the information required to undertake such assessment are judged to be site specific to a particular remediation option. Nevertheless, attempt have been made to clarify the likely significant of the impacts for selected remediation options.

The bioremediation technique enhanced by the addition of BSG was found to changes the soil pH (chapter 4) and improved the removal of the diesel from the soil. Enhanced bioremediation can affect the organic matter and nutrient balance of the soil, which may lead to a change in the balance of the localised existing ecosystem functionality. Although toxicity testing was not carried out in the study to determine the effect of the BSG on the soil, in a study carried out at the University of Sunderland by Ben-Hamed, (2012) to assess alternative agricultural uses of spent grains and investigating the effect of BSG on plant growth, found that after twelve weeks of study the weight of leaves and roots increased with the level of BSG added. The results did not show any deficiency of plant growth as a result of the addition of the BSG that may alter the biological status of the soil but further research is needed to establish the state of the soil biological functions when BSG are added to soil contaminated with diesel oil as this may affect the organic nutrients of the soil.

The impacts of natural attenuation on land include the contaminants remaining in the ambient air for a long time. They are also likely to remain in the sub-surface for longer due to reliance on natural processes. This may make the condition of natural attenuation unpredictable because of the potential for contaminant migration, which may result in secondary emissions heavier than the original impact. In the study here it may impact on the nearby water course, if there is flooding or possible underground water migration. The natural attenuation technique could be compared to bioremediation without the addition of BSG in that both do not have any amendment added but the latter was enhanced through turning the soil and maintaining the water holding capacity of the soil. Whilst their impacts have not been measured for land both has comparable environmental footprints.

The environmental impact of landfill on land is likely to be significant as the physical properties of the soil are altered by excavating the soil and filling the site with new

soil. This is because indiscriminate removal of soil organic matter and organisms changes the biological state of the soil. The new soil may not possess the excavated soils ecosystem functionality. But the use of the site in the study is for housing development and as such replacing with clean soil is unlikely to have a significant impact on ecosystem functionality.

The environmental impact of SVE has not been recorded in the UK possibly as the technique is not commonly used as compared to other countries such as The Netherlands, Germany and U.S. where it is widely used as a mature technology (Defra, 2010). But a few studies have shown that during vapour extraction air injection could lead to dangerous vapour migration to buildings and underground facilities. This impact is not evaluated in the study reported here.

Thermal desorption is a process technology that raises the temperature of the soil which may encourage a temporary increase in biological activity of the soil. But the technique could have the potential to damage the soil structure. This is because a high temperature may impact on the organic matter of the soil and could have a sterilising effect on the biological activity and consequently on the long term localised biodiversity (Defra, 2010).

In the U.K. the first two projects that used this technology were in Teddington, a former gasworks site in which electrical conductive heating, similar to the one described in this project was used in 2006. The other was in Harwell (U.K.) where electrical conductive heating was combined with SVE. According to the Defra Report both projects were successful. However, the present study does not evaluate the impacts of thermal desorption on land but due to the high temperature used to capture the gases there could be a sterilizing effect on the soil, which could stunt biological activity and inhibit ecosystem functionality (Defra, 2010).

6.11 Conclusions

The careful evaluation of remediation methods is important if resources are not to be wasted and environmental and health risks are to be minimised. In the study here environmental impacts of using BSG to augment bioremediation was compared to other remediation methods, such as bioremediation without BSG, landfill, natural attenuation, SVE and thermal desorption.

The results of the CO_2 emissions from bioremediation with and without BSG were monitored over a timeframe and bioremediation with BSG was higher than the emissions from natural attenuation. In the field these values are expected to be the same irrespective of the total timeframe for natural attenuation to be achieved due to the mass balance of CO_2. In addition, because BSG is a biodegradable by-product it is expected to emit CO_2 to the atmosphere if used in bioremediation. Hence the carbon footprints of any bioremediation process using organic waste are expected to be greater than that of natural attenuation. However, if the biodegradable by-product was to be landfilled the CO_2 would still be emitted.

Thermal desorption was the least environmentally friendly technique followed by landfill disposal and SVE as compared to bioremediation with BSG in terms of the amount of CO_2 emitted. The reasons for high CO_2 from thermal desorption and SVE was due to the high energy demands of the technologies. The techniques of thermal desorption and SVE in this study did not generate any CO_2 from the process of remediating the soil as the contaminants were captured, treated and disposed of safely but the implementation incurred high rates of electricity and fuel consumption. This evaluation underscores why all inputs in remediation activities and processes are important and should be calculated during the remediation process.

Landfill disposal also has higher CO_2 emissions when compared to bioremediation with BSG due to the high fuel consumption used by the heavy goods vehicle for the disposal of the contaminated soil and re-filling of the land. This showed that the contaminants were moved from one location to another and the activities involved also generated CO_2 and added to the net emissions of CO_2 from landfill disposal. Landfill is also associated with CH_4 if the contaminated soil is deposited in the landfill due to an anaerobic decomposition of the organic matter and the impact of CH_4 on climate change is 20 times greater than CO_2 over a 100 year period. Landfill is associated with other pollutants that affect local air quality such as NOx, PM_{10}, $PM_{2.5}$, CO, NH_3 and SO_2. The overall consideration of these impacts may make landfill disposal less attractive. In practice these emissions may not be considered, as a typical site of this nature in the U.K. is usually landfilled due to the short time required to remediate the site.

The environmental impact of the GHG's from the results showed that bioremediation

with BSG was lower than thermal desorption and SVE but was associated with different pollutants that affect local air quality. These include pollutants such as NOx, PM_{10}, $PM_{2.5}$, CO, NH_3 and SO_2. Therefore, there is need for future discussion concerning what constitutes the highest environmental footprint between GHG and other air quality emissions during remediation selection process. This has become necessary due to the health effects of these emissions and currently the pollutants are not often considered when choosing the best method for remediating contaminated sites. These local air pollutants are associated with bioremediation with BSG and landfill disposal option and their values in this study are negligible.

Chapter 7

DISCUSSION

7.1. Introduction

In the last decade there has been an increased awareness of the impact of greenhouse gasses on the environment and as such individuals, organisations and governments have come to realise that emissions of greenhouse gasses need to be reduced (Loo, 2009). Extensive research has been carried out aimed at explaining the changes in climate due to greenhouse gasses and to explore the means by which they can be countered. One of the commonly accepted methods to reduce climate change is the reduction of the greenhouse gas emissions (Schers, 2006).

Pollutants such as CO_2, CH_4 and N_2O trap the outgoing infrared radiation from the earth's surface. This process is often referred to as the greenhouse effect, which adds to the net energy input of the lower atmosphere and results in regional and global changes in climate parameters including temperature and rainfall (Rastogi *et al.* 2002). Global climate change has been judged to be correlated with the use of fossil fuel (Ellis and Hardley, 2009) but remediation techniques including landfill disposal, SVE, natural attenuation, thermal desorption and bioremediation are associated with different impacts, which may contribute during the process of removing pollutants from the soil, air and water.

Methods commonly used to clean-up hydrocarbon polluted soil involve heavy engineering methods which typically offer relatively quick-fix solutions and could be expensive with high environmental and social impacts (CL: AIRE, 2007 and Defra, 2010). Historically in the U.K. and elsewhere soils contaminated with hydrocarbons are usually disposed of to landfill. But this technique cannot be viewed as sustainable due to its high environmental footprint. There is also legislation from the European Union and U.K. government which sets targets for the reduction of biodegradable and hazardous wastes going to landfill and there has been a decrease in the number of landfill sites that will accept hazardous waste such as hydrocarbons. This has resulted in fewer landfill sites and higher landfill tax to dispose of contaminated soils which are normally classified as hazardous waste.

As such legislative and economic drivers have driven the need for alternative options

for the clean-up of polluted land and bioremediation is an increasingly popular option. Bioremediation optimizes the biological system already present in the soil and degrades the contaminants to an innocuous end or harmless product (Hamby, 1996). The technique is thought to be safe, reliable and environmentally friendly because it relies on the natural assimilative capacities of the soil to breakdown the contaminants (Nichols and Venosa, 2008).

However, the breakdown process can be slow. As it relies on the indigenous microorganisms to degrade the contaminants there is t a need to improve the natural breakdown processes by the addition of nutrients or other growth limiting co-substrates not normally present in sufficient quantity in the soil. Nutrients normally added could be organic or inorganic. Organic wastes could be a source of both nutrients and microorganisms to improve upon the breakdown of the hydrocarbon polluted soil (Walworth *et al*. 2003). Utilizing biodegradable waste in this manner would divert the waste stream from landfill. BSG is a by-product from the brewery processes with high water and nutrient content (Thomas and Rahman, 2006). It is currently disposed of as an animal feed. However, the amount generated means that the demand for the product is not as high as the volume produced.

However, using organic wastes/by-products, including BSG, to augment the bioremediation process may be associated with potential environmental impacts. These could include leachate from the decomposing organic matter that may percolate through the soil and possible release of microorganisms into the atmosphere (Komolis and Ham, 2006). In addition, the bioremediation process may involve movement of vehicles to transport the BSG from the brewery to the contaminated site and this could result in emissions of CO_2. As such the technique may not be economically feasible or as environmentally friendly as initially thought. In addition, the costs of not developing the land, as the time taken for the bioremediation target to be achieved may be greater, than adopting other quicker methods such as landfill.

In order to achieve sustainable remediation the impacts of a given remediation option should not be more than leaving the contaminants untreated. Hence in the present study it is important to balance the economic, social and environmental impacts of using BSG to remediate contaminated soil with diesel. Since a discussion

of the results obtained has been presented at the end of each chapter, this final discussion will give an overview of the study and draw attention to the main findings.

7.2. Does the addition of BSG improve the bioremediation of hydrocarbon contaminated soil?

The results of both experiments carried out for the study reported here, showed a reduction of 95% in diesel contamination was achieved in 104 days in experiment (2) and 60 days in experiment (1) after the addition of BSG as compared to the treatment without BSG, which recorded 91% in experiment (2) and 72% in experiment (1) respectively.

The microbiological results showed the hydrocarbon utilizing bacteria in soil amended with BSG were appreciably higher compared to those without the BSG. The likely reason for this is that BSG is known to have a high nitrogen content (Thomas and Rahman, 2006) and is a necessary nutrient for bacteria biodegradative activities. It has also been reported in other studies that there are high quantities of nitrogen and phosphorus in organic wastes, with appreciable quantities of nitrogen in BSG (Abioye et al. 2012 and Joo *et al.* 2007) and this can then be a limiting factor in the bioremediation of hydrocarbon contaminated soils (Yang, 2009).

The results of the biological and chemical analysis answer the first question of the research that the use of BSG improved upon the bioremediation of diesel contaminated soil. However, remediation of contaminated land has been previously described as being an expensive and technically difficult process and cost is considered as one of the major drivers of remediation (Euro-Demo, 2006). The assessment of the total cost of using BSG to remediate contaminated soil was carried out. In order to achieve this, a case study site was developed. Pollution at the site was diesel with an approximate volume of 20,000 kg spread over an area of 10,000 m^3 and the contaminants were found on the unsaturated area of the site. The next section discusses the results obtained from the case study site.

7.3 Is the use of bioremediation with BSG economically viable?

In order to calculate the actual cost of using BSG to remediate diesel contaminated soil a case study site was developed. The purpose of remediating the site was to build 300 new homes for residential purposes. The site preliminary conceptual model

in chapter 5 highlights the potential sources of contaminants, pathways and receptors.

To determine the economic costs of using the BSG to remediate the soil all costs associated with the processes were evaluated including direct and indirect costs. There is a need to compare the economic cost of using BSG to remediate the soil taking 'time' into consideration and making comparisons to other remediation options such as landfill, natural attenuation, SVE and thermal desorption, which are used in the U.K. and elsewhere.

The timeframe for the remediation objective to be realised in the field, using BSG has been estimated to be six months (chapter 5) and this timeframe was used when estimating indirect economic costs associated with delayed development at the site.

There are limited studies that address the 'true' cost of remediation of contaminated land, as cost is a site specific issue (Defra, 2010).The true costs of remediation are not often calculated by remediation practitioners because costs relating to one site may not be applicable to other sites meaning that decisions as to the choice of remediation technique to be used may not be based upon accurate and realistic costings.

The cost of using BSG to remediate the soil was calculated including the direct and indirect costs associated with the technique. The direct cost is the actual costs that can easily be quantified because they have market value. Whilst indirect costs could be described as costs that reflect activities that indirectly induce costs during the period of remediation, such as the time scale it takes to achieve the remediation objective and other hidden costs that are not normally considered during the remedial selection process. Categorising costs in this manner will allow remediation practitioners to build safe, durable and economic structures (Tedd, 2001) and make an informed decision on the actual remediation costs as this falls into the ethos of sustainable development.

The total costs of using BSG to augment the bioremediation process in the case study site showed that bioremediation, with BSG was a viable option and cheaper than other remediation methods considered in the study. In addition, the results of the cost model in the study demonstrated how costs are sensitive to certain

conditions and could be a function of the purpose of the site, the technology used and the duration of the remediation works.

However, economic cost is only one strand of sustainability. Hence there is need to investigate the environmental cost of using BSG to augment the bioremediation process.

7.4. What are the environmental costs of bioremediation using BSG?

Historically, the major concerns in selecting a remediation technique in the UK have been costs and feasibility (CL: AIRE, 2006). This is beginning to change with greater appreciation of environmental and social impacts and the consideration of these elements would be valuable in the design of a sustainable remediation project. The case study site presented here compared the environmental cost of bioremediation, with the use of the BSG, with other remediation options such as landfill disposal, natural attenuation,SVE and thermal desorption.

As the bioremediation process relies on the optimization of indigenous microorganisms to break down the contaminants, diesel will be broken down and released as CO_2 and water. This will generate emissions that will affect climate change with global warming potentials. More so, the use of heavy goods vehicles to transport the BSG and the use of heavy machinery for tilling and turning the soil during bioremediation activities will result in emissions to atmosphere.

Whilst finding the most suitable technology for the remediation of a contaminated site, environmental sustainability of the chosen technology is becoming an important consideration. However, it is far more difficult to evaluate environmental costs, as compared to economic costs, due to the difficulties of ascribing financial values to environmental impacts and the inability to arrive at accurate figures. Hence for many remediation projects environmental evaluations are not considered and in situations where evaluation is carried out various values are derived from different assessors, even when the projects are of the same nature. For example a group of practitioners in the remediation industry were surveyed in the U.K., and it was found that most of them evaluated their environmental sustainability differently using various techniques and matrices and they did not adhere to a particular approach (SuRF-U.K, 2010).

Reddy and Adams, (2010) asserted that there is no universally accepted way of

calculating the environmental elements including CO_2 and other environmental footprints and this has led to an array of carbon calculators that have been developed over the past few years creating confusion and inaccurate information. An attempt to measure emissions associated with bioremediation, with the use of BSG, has been carried out in this thesis. A laboratory experiment was conducted to determine the CO_2 associated with bioremediation methods and the measurement of all the environmental impacts including land and water media and the results were compared to other remediation options considered in the study.

Table 7.1 below shows the overall impacts on each of the environmental media.

Media	Overall impacts (most impacted)	Overall impacts (less impacted)
Air pollution	Thermal desorption, landfill and soil vapour extraction	Bioremediation with and without BSG and natural attenuation
Water pollution	Bioremediation with and without BSG and natural attenuation	Soil vapour extraction and thermal desorption
Land pollution	Natural attenuation, soil vapour extraction and thermal desorption	Bioremediation and landfill

Table 7.1 The overall impacts on the three media of air, water and land pollution.

From the results in Table 7.1 air emissions involved the quantitative evaluation of the impacts of the six remediation options and values were ascribed to the emissions and comparisons were made amongst the different remediation methods. It was found that natural attenuation was the most environmentally friendly followed by bioremediation without and with BSG. Thermal desorption was the least environmentally friendly option. SVE and landfill disposal options were higher in terms of environmental cost than bioremediation with BSG. This valuation was based on the amount of greenhouse gases emitted by each remediation technique.

There were other pollutant emissions that affect local air quality and these emissions

were only associated with landfill and bioremediation with BSG, due to the use of heavy good vehicles to transport the diesel contaminated soil and the transportation of the BSG from one location to another. The pollutants include CO, NOx, VOCs and PM_{10}. These emissions are not normally considered in practice during selection, design and implementation of sustainable remediation technologies at contaminated sites and incorporating these elements will pave a way to determine which air pollutants should be considered.

Water and land media were assessed qualitatively without ascribing values to their impacts. This highlights the problem of assigning values to all environmental media. There is a need for accurate costing models that cut across the three environmental media.

The work presented in this study has shown the evaluation of six different remediation methods qualitatively and quantitatively. The evaluation was based on economic feasibility and the environmental cost of implementing the different technologies. A comparative assessment of bioremediation and other remediation techniques showed that bioremediation, with BSG, seems promising as compared to other options due to its low economic costs and reduced environmental impact.

But will these meet the criteria of sustainable remediation without taking into consideration the social strand of sustainability? The next section will introduce this social element.

7.5. What are the social elements of the use of BSG to remediate diesel contaminated soil?

The social element of remediation according to SuRF-UK, (2010) are actions that impacts on human health and safety, ethical equity considerations, impacts on neighbourhoods or regions, community involvement and satisfaction, compliance with policy objectives and strategies and uncertainty. These are regarded as social indicators that should be measured during remediation of groundwater and land contamination.

The social focus with the use of BSG to remediate diesel contaminated land would involve risks to site workers, neighbours and the public from remediation work including hazardous process emissions such a PM_{10} and $PM_{2.5}$, noise, odour, dust,

and chemical exposure of the diesel contaminants (as it was carried out above ground) during excavation and turning of the soil by heavy machinery.

The social impacts resulting from the movement of heavy goods vehicles to deliver the 2,000 tonnes of BSG to the site and the impacts of traffic within the locality should be considered. These impacts according to the guidelines in (SuRF-UK, 2010) should be measured if the remediation activities may have an effect on neighbourhoods. Different remediation technologies are associated with different levels of impact that will affect community satisfaction.

In the study here the social element may also include benefits such as the use of minimal water during remediation and the use of recycled by-product material, which is an alternative option to disposing of the by-product. These benefits and other impacts mentioned above would be compared to other remediation options considered in the study to determine which option is most sustainable when remediating diesel contaminated soil. Hence there is need to evaluate the social elements of all of the remediation methods considered in this study, however, due to time constraints the social strand was not evaluated, but it is an area of concern that should be addressed. The expected social benefits of using BSG to augment bioremediation include:

- The process encourages the re-use of by product material.
- The process diverts BSG from landfill if it is not being used as feedstock.
- Minimize risk to site workers and neighbours through reduced movement of heavy goods vehicles when compared to landfill.
- Noise level is minimal compared to other methods such as SVE and thermal desorption.
- There is a reduction in emissions compared to other options in the study.
- Minimal uses of water compared to other remediation options such as thermal desorption and SVE.

- Land is put into alternative use as the project provides affordable houses and re-generates the area with 300 new homes.

Therefore, sustainable remediation aims to balance the remediation practices, processes, and technologies with the need to use less energy and resources, reduce emissions, without negative impacts on the lives of occupants, neighbours, and ecosystems during the implementation of the remediation techniques (Gimpelson, 2011). Various qualitative and quantitative analytical methods can be used to provide the neccessary information and sustainable remediation methods, which entail balancing multiple factors to create a holistic assessment of each method to select the one that provides the greatest benefit for all.

Chapter 8

CONCLUSIONS

8.1 Introduction

The work presented in this study has shown that the addition of BSG has resulted in the bioremediation of diesel contaminated soil and breakdown of the diesel by 95% (concentration 3,400 mg kg^{-1}) over 60 days and 95% (concentration 68,000 mg kg^{-1}) over 104 days. It also showed that the technique is an economically viable option with reduced environmental costs. A comparative assessment of bioremediation and other remediation techniques in the study showed that bioremediation with BSG seems promising due to its low economic and environmental costs. The evaluation was based on economic feasibility and the environmental cost of implementing the different technologies.

Amongst the remediation options considered natural attenuation was an attractive remediation technique in the study but there was a high economic cost associated with not developing the site coupled with cumulative impacts that would occur during its characteristic long duration. The same can be said of the SVE technique, which has a long duration to achieve the remediation objective and high economic costs due to the likelihood of the SVE system being unable to extract some diesel components. SVE and thermal desorption techniques are technologies that encourage controlled contaminant partitioning between the environmental media in that contaminants are moved from soil to liquid or from liquid to air.

The excavation and disposal option will result in immediate and long term emissions due to emissions from heavy goods vehicles to transport the contaminated soil from one location to another (immediate) and the breakdown of the contaminants in the landfill site (long-term) will generate CH_4. In addition, the impact of CH_4 on climate change is over 20 times that of CO_2 over a 100 year period.

Remediation technologies such as bioremediation have been identified in the study as needing a low energy input and shorter treatment time due to the augmentation of the BSG to speed up the process.

Summary of key findings:

- The economic and environmental impacts of remediating the soil using BSG to augment the bioremediation process is less than the impacts of leaving the site untreated.
- It is a technology that promotes the re-use and utilization of by-product materials such as BSG.
- It is a technique that reduces energy consumption associated with site remediation.
- The bioremediation processes minimize the risk to public health and the environment in a cost-effective manner and at a reasonable time frame compared to other options.
- It minimizes the emission of air pollution and GHGs and minimizes impacts to land and ecosystems.

8.2 Recommendation for future work

There is need for the technology to be transferred to the field in order to demonstrate the practicality of the technique.

Other future work needed includes:

The bioremediation process of using BSG was successful in the laboratory but there is need for field scale trials across a range of soil types.

- There is also a need to obtain accurate quantitative values for environmental impacts for water and land.
- The addition of BSG may bring with it other micro-organisms and nutrients. This needs to be investigated.
- There is a need to investigate the CO_2 released from the BSG itself during the bioremediation process.
- There is need to measure the social element of sustainability in order to have a holistic assessment of the bioremediation process.

- In terms of sustainable remediation there is a need to develop indicators for the social impacts.

The results of this study showed that thermal desorption was the least environmentally friendly option. Bioremediation and natural attenuation were the most environmentally friendly method. SVE and landfill disposal option were higher in terms of environmental costs than bioremediation with BSG. This valuation was based on the amount of greenhouse gas emitted by each remediation techniques. However, the results of the potential impacts on other media, such as land and water, indicate that the six remediation technologies do have environment impacts with variations with each media.

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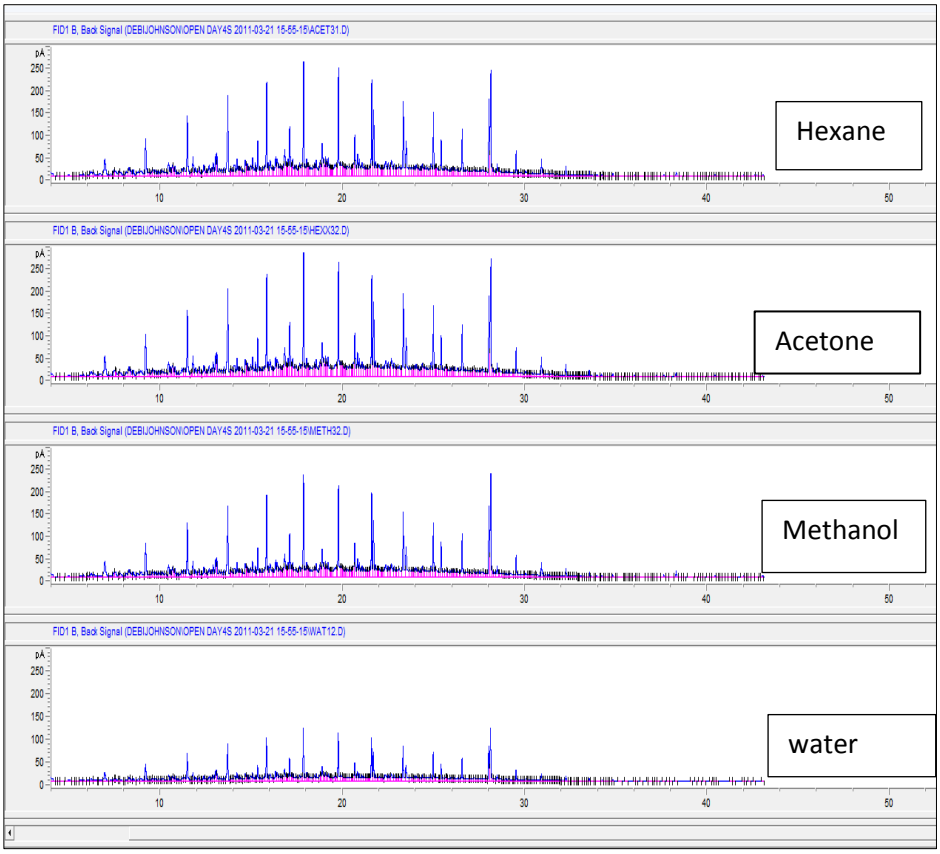
Appendix1



Appendix I: Summary of the Gas chromatographic profiles of three solvent and water put on the same scale and the carbon range measures

Appendix ll:

The soil used was the agricultural soil described in section 3.2.1 above. The experiment was set up with 10 g of soil. This was weighed into a crucible. The crucible was then weighed before being placed on a metal tray and put into an oven that had been pre-heated to the temperature of 105^0C.

The soil was left at this temperature for 24 hours then taken out and allowed to cool in a desiccator. The crucible plus the soil was then reweighed. The following method was adopted to calculate the WHC for soil and brewery spent grain:

Fresh weight of sample= (Weight of crucible + fresh soil) – weight of crucible

Dry weight of sample= (weight of crucible + dry soil) – weight of crucible

Dry matter per cent content (DM %) = (Dry weight of soil/fresh weight of soil) x 100%

Moisture per cent content MC%= 100-DM

Once the moisture content of the soil has been determined the water holding capacity can be found by using the following procedure.

A series of beakers was suspended from clamps. These were set up in replicate for soil and grain in addition to 2 blanks. Attached to the mouth of each beaker was a short length of rubber tubing (8 cm). Each tube has a clip which is tightly closed to prevent it from leaking.

Each beaker was then lined with wool glass. Then 50 g of soil per replicate was weighed and transferred into the beaker. The blank samples contain only wool glass. A 50 cms^3 measuring cylinder was placed beneath each beaker. 50 cms^3 of water was then poured into each funnel and left for more than 30 minutes to saturate the soil. After 30 minutes the clips to the tube were loosened to allow the water to drain from the beaker down to the cylinder which was held beneath the funnel. After 30 minutes the final volume of water from each cylinder was collected. The same procedure was repeated for grain.

Therefore, the volume of water retained by the soil and grain is calculated from 50 – (volume water retained by glass wool + volume water collected) ml = A

The volume of water retained by the glass wool would be the initial 50 cms^3 of water poured into the beaker = 50 cms^3 – the volume of water collected from the blanks.

To calculate soil and grain WHC cms^3 water held at 100% WHC per 100 g oven dried and soil or grain) 2A + MC% = WHC (cms^3 100 g fresh soil and grain) = B cms^3.

Then: (B cms^3/ soil or grain DM) x 100 = cms^3 of water held by 100 g oven dried soil or grain at 100% WHC.

The WHC of soil and spent grain used in the study was calculated separately below:

Soil and grain water holding capacity

Soil water holding capacity

Weight of soil taken (g)	Weight of soil after drying (g)
10	1.87
10	1.83
10	1.83
Average dry weight of soil	1.84

To calculate the DM%

DM% = (Dry weight of soil/Fresh weight of soil) X 100

DM% = 1.84/10 X 100 = 18.4%

Then Moisture content MC% = 100 – DM

MC% = 100 – 18.4 = 81.6%

To calculate the soil WHC (cms^3 water held at 100% WHC per 100 g oven dried soil)

2A + MC% = WHC (cms^3 100 g^{-1} fresh soil) = B cms^3

To calculate 'A'

50g of soil was weighed into a beaker and 50 cms^3 water added to the surface of the soil. Between the soil and the base of the beaker was placed glass wool. The water trapped by the glass wool constitutes the blank measurement.

Run water in a beaker with glass wool without soil (control)

Blank 1 = 48 ml

Blank 2 = 47 ml

Average of the blanks = 47.5 cms^3

Then

Passing water in beaker 1 = 29 ml

Passing water in beaker 2 = 23 ml

Passing water in beaker 3 = 25 ml

Average of run water = 25.7 ml

To calculate A

The volume of water retained by the soil was calculated from: 50 – (volume of water retained by the glass wool + volume water collected) cms^3

Water retained by soil 'A' = 50 – (2.5 ml + 25.7 ml) = 21.8 cms^3

Where the volume of water retained by the glass wool (glass wool) = 50 ml – the volume of water collected from the blank, which was: 50 – 47.5 = 2.5 cms^3

Therefore: 2A + MC% = WHC (cms^3 100 g-1 fresh soil) = B cms^3

B ml = 2 (21.8) + 81.6 = 125.2 cms^3

Then WHC = B cms^3/ soil DM X 100 =

125.2/18.6 = 6.73 cms^3 100 g of soil

125.2/18.6 X 100 = 673%

i.e 100 g of dry soil would contain 673 g of H_2O

Total mass = 673 + 100 = 773 g

H_2O % = 87.06% or 12.94 dry matter content.

Or

8706 cms^3 per kg = 870%.

Dry weight and water holding capacity of spent brewery waste:

- Dry matter content (DM) spent grain:

Grain taken for each: 50 g

Weight of brewery spent grain taken (g)	Weight of brewery spent grain after drying (g)
50	28.16
50	28.1
50	27.6
Average dry weight of brewery spent grain	27.95

Dry matter per cent content (DM %) = (Dry weight of grain / fresh weight of grain) X 100%

DM% = (27.95/50) X 100% = 55.9%

Moisture MC% = 100 – DM = 100- 55.9 = 44.1%

- Water holding capacity (WHC) of grain:

To calculate the brewery spent grain WHC (cms^3 water held at 100% WHC per 100 g oven dried brewery spent grain)

2A + MC% = WHC (cms^3 100 g-1 fresh brewery spent grain) = B cms^3

To calculate 'A'

50g of brewery spent grain was weighed into a beaker and 50 cms^3 water added to the surface of the soil. Between the brewery spent grain and the base of the beaker was placed glass wool. The water trapped by the glass wool constitutes the blank measurement.

Blank already calculated to be 47.5 above

Passing water in beaker 1 = 44 ml

Passing water in beaker 2 = 44 ml

Passing water in beaker 3 = 44 ml

Average of run water = 44 ml

The volume of water retained by the grain was calculated from: 50 – (volume of water retained by the muslin cloth + volume of water collected) cms^3 = A which is 50 – (2.5 ml + 44 ml) = 3.5 ml.

The volume of water retained by the glass wool = 50 ml – the volume of water collected from the blanks = 50 – 47.25 = 2.5 cms^3

To calculate grain WHC (cms^3 water held at 100% WHC per 100 g oven dried grain) 2A + MC% = WHC (ml 100 g^{-1} fresh grain) = B ml

B ml = 2 (3.5) + 44.1 = 51.1 cms^3 Then

B ml / grain of DM X 100 = cms^3 of water held by 100 g oven dried grain at 100% WHC where

51.1/55.9 = 0.9141

WHC = 51.1/55.9 X 100 = 91.41%

i.e 100 g of dry brewery spent grain would contain 91.41 g of H_2O

Total mass 91.41 + 100 = 191.41 g

H_2O% = 47.76% or 52.24 dry matter content.

or

4776 cms^3 per 1 kg of brewery spent grain or 478%

Appendix III

Diesel used during tilling/turning of the soil during bioremediation with brewery waste

Tilling the soil = 70 litre per day
First month tilling = 3 times
every month for 5 month = 2 times
The litres for first month = 210
The litres for 5 month = 700
The total litres for bioremediation with brewery waste = **910**

Diesel used during tilling/turning of the soil during bioremediation without brewery waste

Tilling the soil = 70 litre per day
First month tilling = 3 times
every month for 8 month = 2 times
The litres for first month = 210
The litres for 8 month = 1,120
The total litres for bioremediation without brewery waste = **1,330**

Table 6.1: showing Calculation of litre of diesel used during tilling/turning of the soil during bioremediation with brewery waste and without brewery spent grain.

Appendix IV

Conceptual Site RISK ASSESSMENT

TASK

Remediation of diesel contaminated site in Sunderland (case study site) for six different remediation methods including natural attenuation, landfill disposal option, soil vapour extraction, thermal desorption and bioremediation with and without brewery spent grain

PRINCIPAL HAZARDS

Risk could arise due to the following hazards

- Collision with moving vehicles used during remediation activities.
- Migration of the pollutants to nearby water course and underground water during remediation work- water contamination
- Emissions from hazardous waste such diesel from the soil – air pollution.
- Risk of damaging underground pipe or utility services as a result of excavating the soil or installing machines into the ground.
- Risk of dealing with volatile compounds
- Risk of environmental activist stopping the work or community disturbance

PERSONS AT RISK AND ENVIRONMENTAL RISK

- Workers on site
- General public
- Ground water
- Surface water
- Air pollution

SIGNIFICANT RISKS

- Injuries due to collision with moving vehicles during remediation
- Air pollution due to release of CO_2 and other air pollutants.
- Risk due contaminants migration to surface and underground water.
- Damage to underground pipes and utilities
- Risk of explosions of volatile compounds- diesel

CONSEQUENCES

- Serious water pollution, which is harmful to human, animal and water life.
- Vehicle accidents could result to serious injury and death
- Hazardous chemical such as diesel which is carcinogenic

CONTROL MEASURES (for remediation methods if there are available)

- Trenches are dug around the contaminated site to collect leaching during bioremediation with and without brewery spent grain.
- Collected leaching is treated and monitor all time
- For soil vapour extraction and thermal desorption preventive measured are in place to avoid underground water contamination by using state of the heart technology. In addition nitrogen is added to capture gases to prevent risk of explosion (during treatment and off-gas facility).
- Permission will be seek from local authority to closed the designated site and road sign are put on site to warn of any danger especially from heavy good vehicles.
- Air samples from thermal facility and soil vapour off-gas treatment plant are checked to make sure meet regulatory requirement.

Appendix V

Electronic resources (internet)

URL-1 Soil phosphatase hand out (online) Available from:

http://www.envsci.rutgers.edu/~phelps/handout.pdf.

(Accessed on 29/09/2009)

URL-2 Introduction to waste hierarchy (online) Available from:

http://www.theengineer.co.uk/assets/getasset.aspx?liAssetID=25331.

(Accessed on 12/09/2009.)

URL-3 Waste strategy for England (WSFE) (2007) Available from:

: www.defra.gov.uk (Accessed on 05/10/2010).

URL-4 Formulation of R2A agar (online) Available from:

http://www.bd.com/ds/technicalCentre/inserts/R2A_agar_Davis.pdf

(Accessed on 10/08/2009)

URL-5 Formulation of Minimal agar (online) Available from:

http://www.bd.com/ds/technicalCentre/inserts/Minimal_agar_Davis.pdf

(Accessed on 10/08/2009)

URL-6: 17 Centers for Disease Control and Prevention. *Toxicological Profile for Fuel Oils.* Available from: http://www.atsdr.cdc.gov/toxprofiles/tp75-c3.pdf (Last accessed: 08 December 2012)

URL- 7:

http://www.mpcd.info/index.php?option=com_content&view=article&id=443&Itemid=177

URL-8: EPA: overview of greenhouse gas: Methane emissions (Available:

http://epa.gov/climatechange/ghgemissions/gases/ch4.html)